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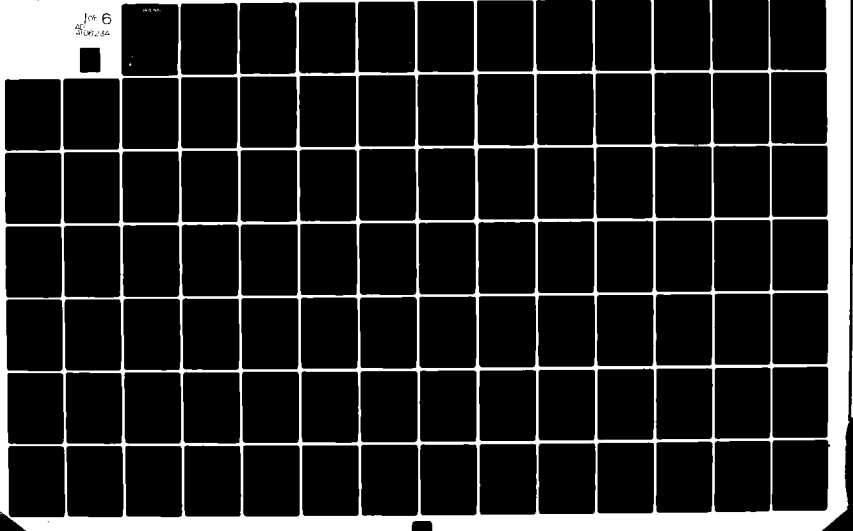

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BIOLOGICAL EFFECTS OF LASER RADIATION

Final Scientific Report - Volume I
(Review of the Literature on Biological Effects of Laser Radiation—to 1965)

Samuel Fine
Edmund Klein

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Submitted October 17, 1978
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Biological Effects of Laser Radiation; Electron Spin Resonance; Spectroscopy; Microscopy: Tissue and Cell Culture Interaction; Biochemical Studies and Macromolecular Transformation; Normal Animals; Tumors; Mechanisms; Laser Radiation Hazards; Flash Lamp Hazards; Electrical Hazards; Scattering; Polarization; Non-Linear Effects.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Research on biological effects of laser radiation to 1965 is reviewed. Laser production of free radicals, use in microscopy and holography, and tissue and cell culture effects are discussed. Possible beneficial transformations in macromolecular preparations are detailed. Effects on normal animals together with gross and microscopic studies are described. Benefits and hazards of applicability and relationship to animal tumor models are presented. Ophthalmological uses and hazards are covered. Protective systems, limitations in applicability of systems current at that time to ophthalmology and in

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experiments, are indicated. Possible use in dentistry and in entomology are reviewed. Mechanisms include consideration of thermal and non-thermal models, pigmentation, interfaces, steam production, pulse duration, non-linear and sonic waves, bubble production and collapse, charged particle production, hypersonic waves, and radiation scattering. Problems associated with measurement of temperature and pressure and methods for carrying out such measurements are presented. A section on non-linear effects discusses second harmonic generation in laser systems and production in target sites, Raman scattering and possible production of ultraviolet radiation. Effects and hazards are considered. Sections on gas lasers, flash lamps, electrical hazards and scattering are included.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

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BIOLOGICAL EFFECTS OF LASER RADIATION
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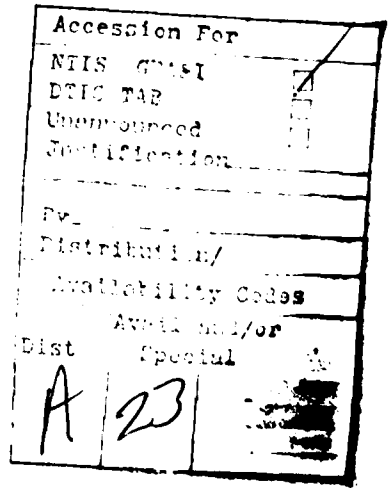

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The report presented in the following pages constitutes the efforts of a group consisting of individuals with training in the various scientific disciplines. Although major contributions to the various sections have been credited on the previous page, it should be recognized that many contributions have been made by these individuals to chapters other than those to which they are major contributors. A number of individuals who have contributed to various sections may have been inadvertently omitted. An attempt will be made to rectify this omission in the final report.

Due to the time duration from initiation of the contract to preparation of this report, the mathematical derivations in various sections, particularly if they are original, should be considered as not being in final form. Some of the models developed for biological systems will require refinement.

The authors wish to thank the many individuals who have contributed their time and effort towards this report. In particular, they wish to thank Miss Angela Reid, Mrs. Patricia Brown, Miss Grace Wood, Miss Joan Liquori, Miss Sandra Taylor, Miss Avril Gozna, and Miss Linda Law for their devoted secretarial assistance.

Note on Content

This review of the literature on biological effects of laser radiation was submitted to the U. S. Air Force Missile Development Center, Air Force Systems Command, as part of a management study. It contains a review of our studies up to and including 1965, which were supported by the U. S. Army Medical Research and Development Command. In addition, it includes a review of work by others.

It is being submitted as Volume 1 of the final scientific report to provide information concerning our research under the contract to 1965. It should be noted that this volume was completed at that time.

Chapter 20, Hazards Due to Light Scattering, was extensively revised and corrected in subsequent volumes. Since it is not of major significance in regard to review of our work to 1965, the chapter was not revised for purposes of this report. A revised version, as submitted in Part III of the original report in 1966, is included in the addendum.

FREE RADICALS AND ELECTRON SPIN RESONANCE STUDIES

Several methods have been used to study free radicals. Potentiometric titration was discussed by Michaelis (1,2). Magnetic susceptibility techniques are reviewed by Brill (3). The appearance of the radical can often be detected colorimetrically or spectroscopically (2,4).

Electron spin resonance techniques are presently the most common methods of investigation (5,6 - 9,11). The method is based on exploitation of the characteristic feature of the free radical: the unpaired valency electron. The unpaired valency electron has both angular momentum and a magnetic moment. A free electron, itself, should give a single, sharp absorption line. However, the unpaired electron in free radicals will be associated with one or more nuclei. The effect of these nuclei is to perturb the local magnetic field in which the electron moves. For example, the observed effect of a proton coupled with the electron is to produce splitting of the single resonance line; two protons will result in a triplet spectrum; a nucleus of higher order in a more complex spectrum. Other factors modify the spectrum obtained. The nature of the free radical consequently cannot be deduced from its ESR spectrum alone.

Free radicals are normally present in many types of tissue. They are considered as essential intermediates in many metabolic pathways (12,10) and have been implicated in carcinogenesis and aging (7). They occur during a number of enzymatically catalyzed reactions and are of significance in photosynthesis (13).

Electron spin resonance signals have been obtained from liver, kidney, heart, lung and adrenal of several species (14). Although not universally

valid, metabolically active tissue such as liver appears to possess a higher free radical content than less metabolically active tissue. A relationship of free radical content to mitochondral concentration has been considered. The variation in free radical concentration in the rat liver with age has been studied. Melanin containing tissues yield an ESR signal, due to the melanin. The melanin has been considered as a semiconductor with bound protons serving as electron traps. It has been suggested that melanin acts as a trap for otherwise harmful free radicals produced.

A reversible threefold increase in unpaired electron concentration, on irradiation by visible light of aqueous suspensions of eye malanin, indicates that melanin may be of importance in the photoreceptive processes although not specifically in image formations. Artificial and dried melanin did not show this effect.

In studies on heart muscle mitochondria the definite oxygen and temperature sensitive ESR signal observed is considered as due to succinic dehydrogenase. Signals have also been measured in other flavoprotein enzymes, including dehydro-orotic dehydrogenase and DPNH-cytochrome reductase.

Free radicals are of significance in photosynthesis. The literature in this field is vast, and is reviewed by Blois and Weaver in Photophysiology, edited by Giese (13). One ESR signal observed on illumination decays rapidly after the light is turned off, a second residual signal may remain for minutes or hours. The rapid signal is considered as associated with chlorophyll, and probably coincides with the primary process of photosynthesis. The origin of the residual or slowly decaying signal is not well defined.

In some cases, as in the study of some flavoprotein enzymes, the appearance of an ESR signal does appear to be correlated with the appearance

of certain optical absorptions that are apparently characteristic of flavin free radicals (12). This has been considered as due to the fact that the riboflavin may have accepted an electron, but is still in close contact with the donor molecule. Observations of this type suggest that complete reliance should not be placed on ESR studied for determination of the presence of free radicals

The purpose of the foregoing discussion is to indicate that numerous studies have been carried out on free radicals, particularly using electron spin techniques. Elucidation of the presence of free radicals, their characterization and quantitation required considerable study.

The studies by Derr, Klein and Fine (15,16) on detection of free radicals by electron spin resonance following laser irradiation were carried out to determine whether free radicals could be produced on laser irradiation. Characterization of the free radicals was left to future investigation.

An ESR signal was obtained in black mouse skin and an enzyme preparation, fibrinolysin, following laser irradiation at 6943 Å. The g value of approximately 2.002 indicated that the signal was probably due to the presence of free radicals. The continued presence without deterioration of signal in irradiated black skin warmed to room temperature for 24 hours, would indicate that the free radicals produced were stable free radicals. The essential difference between the white mouse skin, in which no ESR signal was obtained, and black mouse skin is the presence of melanin and a melanin precursor system. Consequently, the signal observed may have been due to the production of free radicals in melanin (or in the melanin precursor system). Since all tissues that contain melanin can yield an ESR signal (12), the undetectability of free radicals in the unirradiated black mouse skin requires further

explanation. It may be due to the fact that the concentration of melanin free radicals in unirradiated black skin was below the threshold detectability of the ESR system used.

The actual frequency of the spectrometer used by Derr et al. appears to have been of the order of 30 gigacycles (15). For biological studies, this frequency may prove preferable to the usual 10 gigacycle frequency unit. It may have been desirable to provide more information concerning the ESR spectra shown of the various tissues, such as whether the figure represented tissue that was warmed previous to measurement or not. Further characterization of the free radicals produced is desirable, and comparison with those observed on heating and ultraviolet irradiation indicated. Plucking of hair in the black mouse skin previous to irradiation may assist in determining whether melanin is of significance in the production of the free radical. The melanin granules may act as an energy absorption or transfer agent with production of free radicals in adjacent tissue. If separation of melanin from the remainder of the tissue can be achieved with little trauma, this may assist in determining the site or production of the free radicals.

Further studies on smaller molecules may assist in determining the nature of the free radical produced on laser irradiation, and possible the site of photon interaction with the molecule. It is possible that ESR spectra will be obtained on laser irradiation, whereas spectra may not be observed on low level irradiation at the same wavelength. This can occur for several reasons. A certain number of free radicals must be present to be detectable. If the decay time is rapid, then high intensity radiation may provide a number which are detectable, whereas low intensity radiation

would not provide the same concentration. Two photon processes dependent on intensity may result in free radical formation, otherwise unobservable. At low radiation intensity levels, the effects are due to photon-molecule interactions. At high intensity levels, the effects may be due to the production of ultrasonic or hypersonic frequencies, which can result in the production of free radicals. Some free radicals may be due to localized elevation of temperature. The effect of general elevation of temperature of the medium can be reduced by cooling the sample prior to and during irradiation.

REFERENCES

1. Michaelis, L., Free Radicals as Intermediate Steps of Oxidation Reduction, Cold Springs Harbor Symp. Quant. Biol. 7:33-46, 1939.

2. Chance, B., Free Radicals and Enzyme-Substrate Compounds: A Tribute to Leonor Michaelis, in Free Radicals in Biological Systems, edited by M.S. Blis, Jr., H.W. Brown, R.M. Lemmon, R.O. Lindblom, and M. Weissbluth (New York: Academic Press, 1961) pp. 1-16.

3. Brill, A.S., The Detection of Free Radical Intermediates in Biochemical Reactions by Their Magnetic Susceptibility, in Free Radicals in Biological Systems, edited by M.S. Blois, Jr., H.W. Brown, R.M. Lemmon, R.O. Lindblom and M. Weissbluth (New York: Academic Press, 1961) pp. 53-74.

4. Beinert, H., and Sands, R.H., Semiquinone Formation of Flavins and Flavoproteins, in: Free Radicals in Biological Systems, edited by M.S. Blois, Jr., H.W. Brown, R.M. Lemmon, R.O. Lindblom, and M. Weissbluth (New York: Academic Press, 1961) pp 17-52.

5. Ingram, D.J.E., Free Radicals as Studied by Electron Spin Resonance, (New York: Academic Press, 1958).

6. Free Radicals in Biological Systems, edited by M.M. Blois, Jr., H.W. Brown, R.M. Lemmon, R.O. Lindblom, and M. Weissbluth (New York: Academic Press 1961).

7. Sogo, P.B., Tolbert, B.M., Nuclear and Electron Paramagnetic Resonance and Its Application to Biology, Advan. Biol. Med. Phys. 5: 1-32, 1957.

8. Smaller, B., Electron Paramagnetic Resonances Studies of Biological Interest, Advan. Biol. Med. Phys. 9:225-269, 1963.

9. Bersohn, R., Electron Paramagnetic Resonance of Organic Molecules, in Determination of Organic Structures by Physical Methods, Vol. 2, edited by F.C. Nachod and W.D. Phillips (New York: Academic Press, 1962) pp 563-616.

10. Weissman, S.I.,"Electron Paramagnetic Resonance," Comprehensive Biochemistry III, Florkin, M., and Stotz, E.H. (Ed.), (Amsterdam: Elsevier, 1962), pp. 189-208.

11. Boag, J.W., "Electron Spin Resonance in Biology," Radiation Effects in Physics, Chemistry and Biology, Ebert, M., and Howard, A.,(Ed.) (Chicago, Ill.: Year Book Medical, 1963), pp. 194-214.

12. Isenberg, I., "Free Radicals in Tissue, "Physiol.Rev., 44:487, 1964.

13. Blois, M.S., Jr., and Weaver, E.C., "Electron Spin Resonance and Its Application to Photophysiology," Photophysiology, I, Giese, A. C. (ed.), (New York: Academic Press, 1964) pp 35-63.

14. Commoner, B., and Ternberg, J.L., "Free Radicals in Surviving Tissue," Proc. Natl. Acad. Sci., U.S., 47:1374, 1961.

15. Derr, V.E., Klein, E., and Fine, S., "Presence of Free Radicals in Laser Irradiated Biological Specimens by Electron Spin Resonance," Appl. Optics, 3:786, 1964.

16. Derr, V.E., Klein, E., and Fine, S., "Free Radical Occurrence in Some Laser Irradiated Biologic Materials,"Federation Proc, 24 (1) Pt III, Suppl. 14:S-99, 1965.

Lasers in Spectroscopy

Two distinct spectroscopic techniques have been used by spectroscopists (1). The high power densities obtained with a focused Q-switched laser are being used in emission spectroscopy to vaporize microscopic amounts of samples for elemental analysis. On the other hand, the intense, highly monochromatic laser beam is being employed as a light source for Raman spectroscopy to study molecular structure. These two uses of lasers in spectroscopic analysis have been sucessful because of the rapid progress made in laser technology to date.

The use of the Laser Microprobe in spectrochemical analysis of the elements is based on the ability of a laser to vaporize material on which it is focused (2,9).This was observed by W.S. Boyle (3), who demonstrated the formation of a luminous plume when a graphite target was irradiated with a focused ruby laser. Similar plumes have been reported following the laser radiation of metals (4) and biological systems (5), and many studies have been, and are being conducted on the nature of this plume by investigators representing several and varied points of view (5,7,8).

In 1962 Brech and Cross (9) developed the "Laser Microprobe", one of the first practical laser applications. The instrument designed by Brech et al. used a small ruby laser focused through a microscope with a 25x, 0.25 NA objective. A sample placed at the focal point of the laser was irradiated, the high energy density vaporizing a small portion of the unknown material, the vaporized material forming the characteristic plume. The plume, containing atomic gases of the sample, was then further excited by a 1 to 2 kv arc between cross firing carbon electrodes placed slightly above the sample (1). The resultant spectrum was recorded photographically by a spectrograph. This original microprobe has gone through several changes in design and operational

improvements including the incorporation of a 2-3 MW, electro-optically, Q-switched laser (10) and more recently the substitution of a 10 MW passive Q-switched laser.* The latter change was accompanied by the substitution of air spaced complex lenses for cemented ones (11).

This system has been used in its various evolutionary phases for a number of spectrochemical analysis of various tissues (11,15), in addition to metallurgical and geochemical studies (10). The first report of tissue analysis with the laser microprobe unit was by Rosan et al. (12). This group analyzed rat brain tissue, sliced to 100μ in a cryotone, dried stained 100μ sections of elastin, and two types of calculus with the microprobe. Four sections of rat brain were analyzed and showed to contain various amounts of Mn, Fe, Cu, Zn and Ca (12). Three 100μ sections of pancreas were analyzed after staining and produced similar spectra, except the pancreatic septum which contained Be in addition to the other five elements (The Fe line and a single faint Zn line in pancreatic acini is very similar to that in the background spectrum). A 12μ section of ligamentum nuchae elastin was analyzed and probably contained Cu, Ca and small amounts of Fe and Zn, while unsectioned, unmounted, unfixed pearly and hemorrhagic periocontal calculus produced strong lines of Ca, as well as lines of Cu, Zn and Fe, attributed to blood by the authors (12). No attempt was made to obtain quantitative data; although, the authors believe relative concentrations can be determined to ± 20%. The authors state that a crater size of 50μ and 10^{-7} gm. sample,can be obtained. This seems to be a very small diameter in view of the results obtained by researchers working with the laser microbeam (see Laser Microbeam Section of this report). Many spectral lines were, of course, not analyzed. Information concerning the grating used, the spectrograph speed and the slit widths employed would have been of interest. In a later report spectrograph speed is given as f/22 (13),

in a Wadsworth mount. Reproducibility presents a problem, particularly since the laser pulse is difficult to reproduce (14). The advantages of this laser spectrograph over standard emission spectroscopy for in vitro sections, particularly prepared sections requires further consideration.

Rosan, Brech, and Glick (14,15,16) have discussed some of the problems encountered in the evolution of this new technique and possible solutions to these problems. A commercial plastic for the sample holder is now in use to avoid contamination encountered with glass slides (11). The recent development of internal standard methods for quantitative analysis of histologic sections has been attempted (14). The method consists of monitoring the Ag in Kodak 649-GH spectrographic film, which has a uniform distribution of Ag (1.5%) in a gelatin matrix (13). Sensitivities of the order of 10^{-11}M (Mg^{++}) to 10^{-9}M (Co^{++}) can be obtained. This method was required to measure Ca and Mg in frozen dried sections of human pylorus, 30μ in thickness (14). However, the 10% to 20% variance obtained for curves of Ca and Mg in concentrations of 10^{-7} to 10^{-12}M or 0.02 meg/l would seem to indicate that further development is required before the laser microprobe can be considered a quantitative tool (14). It is possible that better quantitative results may be obtained using photoelectric detection, rather than photographic methods.

The laser microprobe has been used for a limited number of in vivo spectrochemical analyses (14,15). Rosan, et al. gave no indication what elements were detected in their studies (14). Preliminary in vivo spectrochemical studies by Fine and Klein on the skin of normal intact black and white mice and on melanomas in mice, indicated the presence of Fe, Mg, Si, Cu, Ti, Ag and Ca (15). The relative position of the corresponding carbon electrodes had to be modified to obtain satisfactory results. Detection of various

elements *in vivo* on a clinical basis is possible, but the long term effects of Q-switched laser radiation must be considered before implementation of the method (17).

The laser microprobe may be of value in studies on calcified tissues, bone and teeth (13,18). Since the laser can volatilize a micro area (50μ in diameter by 25μ in depth) of bone or tooth without difficulty (18), determination of inorganic elements can be carried out without previous preparation. Studies have been initiated by Sherman et al. on the concentration of inorganic elements present in various microregions of normal and carious teeth, of supragingival and subgingival calculus, and cortical and trabecular bone (18,19,20).

The comparative study of enamel, dentin, carious dentin, supragingival calculus, subgingival calculus, cervical cementum and apical cementum showed both a qualitative and a relative quantitative difference in the elements present in the various regions (18,20). Of interest was the higher concentration of P and Mg found in carious dentin than in normal dentin. The findings of relatively high concentration (1-10%) in one sample of carious dentin and at lower concentrations in all samples of apical cementum is not well understood. This was also observed in *in vivo* spectroscopy (15). The capability of analysis of microquantity samples of calculus which previously required sample pooling was of interest. Zinc was present in all subgingival calculus analyzed but in only 1 sample of supragingival calculus. However, information as to the number of samples analyzed from each area to establish the significance of these findings would be desirable. Included in these studies were analyses of the rat mandible, which discloses a variation in the phosphorus and magnesium concaentration between cortical and trabecular bone. Variations in the aluminum content of the gingiva, and in the magnesium and silica concentrations in the osseous tissues were found during wound healing in dogs (18,19,20). That

these results, obtained from specific samples are representative of the average amount of the elements found in the tissue requires further study.

Studies by Lithwick, Healy and Cohen (21,22) on 100μ thick, undecalcified cross sections of dog femur and human costochondral junction are of interest in evaluating the laser microprobe. Ca, P, Mg, Al, Cu, Si, Ti, Zn, F, N, and trace amounts of Y were detected (21,22).

Old and new osteons, interstitial lamellae, hypertrophic cell zone and proliferating cell zones of cartilage were analyzed. Sixteen to thirty determinations per bone section were made on the average, and the data were analyzed with reference to Standh"s wet microchemical analysis of Haversian Systems for phosphorus, nitrogen, and calcium to Haumont's analysis of bone for zinc.

The elements Mg, Al, Cu, Ti, Y and Si were consistently found in higher concentration in young bone than in old bone (21). The authors were unable to explain the absence of Pb or the low concentrations of P. They considered the high concentration of the elements Al, Si, Ti, Cu and Zn in young osteons as compared either to old ones or to interstitial lamellas. To be of interest, the authors indicated that extreme care in handling the sample was necessary to prevent contamination, which of course affects the accuracy of the technique.

These authors indicate that established standards are necessary for calibration, if the technique is to be considered quantitative. Some of the biological studies reported would have benefited by further information conc erning the spectrograph used. The problems associated with obtaining reproducable Q-switching require investigation.

The spectra published by Ferguson and Nicholls (23) indicate that a sufficiently intense spectrum can be obtained from the plume of a single

Q-switched pulse, and a fast, high quality spectrograph, such as a Hilger f/4 is used (24). Spectrochemical analysis, using a Q-switched ruby laser as the only source of excitation (without cross firing electrodes) has been reported. A degree of reproducibility high enough to place laser excitation on a par with A-X or D-C arc methods for quantitative analysis were obtained. Ferguson (2,3,7,25) and Howe (26) have observer a number of molecular bands in spectra using only laser excitation.

REFERENCES

1. Brech, F., Applications of Lasers to Analytical Chemistry (a) Atomic "Spark Emission" Spectroscopy (b) Raman Spectroscopy, Abstracts 149th meeting, Am. Che. Soc., Detroit, Mich.

2. Levine, Albert, K., Lasers, American Scientist 51 (1):14-31, March 1963.

3. Schawlow, A.L., Optical Masers, Sci. Ann. 204:52, 1961.

4. Linlor, W.I., Some properties of plasma produced by laser giant pulse, Phys. Rev. Letters, 12: 383-385, 1964.

5. Fine, S., Klein, E., Scott, R., Seed, R., Laser Radiation in the Syrian Hamster, Skin, II:43, Feb. 1963.

6. Ready, J.F., Effects due to absorption of laser radiation, J. Appl. Physics, 36:462, 1965.

7. Mentall, J.E., and Nicholls, R.W., Temperature Measurements on Laser - Produced Flames, presented at spring meeting Am. Phys. Soc. meeting, Wash. D.C., April 27, 1965.

8. Fine, S., Nowak, W., Hansen, W., Hergenrother, K., Scott, R.E., Donoghue, J. and Klein, E., Measurements and Hazards on Interaction of Laser Radiation and Biological Systems, NEREM Record, 1964.

9. Brech, F. and Cross, L., Optical Microemission Stimulated by a Ruby Maser, Appl. Spectroscopy 16:59, 1962.

10. Maxwell, J.A., The Laser: As a Source in Emission Spectroscopy, Chem. in Canada, April 1963.

11. Rosan, R.C., Brech, F. and Glick, D., Spectrographic Analysis of Nanogram Samples by Improved Laser Microprobe Technique, Fed. Proc. 23:174 (1964).

12. Rosan, R., Healy, M., McNary, W., Jr., Spectroscopic Ultramicroanalysis with a Laser, Sci. 143 (3589):236-237, 1963.

13. McNary, W.F., Rosan, R.C. and Healy, M.K., Abstr. 3rd Boston Laser Conf. 1964.

14. Rosan, R., Brech, F., Glick, D., Current Problems in Laser Microprobe Analysis, Fed. Proc. 24 (1):126-128, 1965.

15. Fine, S. and Klein, E., Biological Effects of Laser Radiation, in Advances in Biological and Medical Physics (in press).

16. Rosan, R., Glick, D. and Brech, F., Progress in Laser Microprobe, Emission Spectroscopy, Fed. Proc. 24:542, 1965.

17. Fine, S., Klein, E., et. al., Interaction of laser radiation with biologic systems, I. Studies on interaction with tissues, 24, 1, (suppl. 14) pt. 3, s35-45, 1965.

18. Sherman, D.B., Ruben, M.P., Goldman, H.M., The Application of Laser for the Spectrochemical Analysis of Calcified Tissues, Ann. N.Y. Acad. Sciences, 122:767, 1965.

19. Goldman, H.M., Ruben, M.P., and Sherman, D.B., Application of laser spectroscopy for Qualitative and Quantiatative Analysis of Calcified Tissues, Oral Surg., Oral Med., Oral Pathol. 17, 102 (1964).

20. Sherman, D.B., Ruben, M.P., and Goldman, H.M., Abstr. 3rd Boston Laser Conf. 1964.

21. Lithwick, N., Healy, M., Cohen, J., Microanalysis of Bone by Laser Microprobe, Surg. Forum 15:439-41, 1964.

22. Lithwick, N.H., Cohen, J., and Healy, M.K., Microanalysis of Bone by Laser Probe, Biomedical Laser Conf., Laser Med. Res. Found., Boston, Mass., June 1965.

23. Ferguson, H.I.S. and Nicholls, R.W., Some Further Aspects of Laboratory Atrophysics and Space Science, Can. Aeronautics and Space J., 10: 1964.

24. Mentall, J.E. (Personal communication)

25. Ferguson, H.I.S., Mentall, J.E. and Nicholls, R.W., Laser Excitation of Powdered Solids, Nature 204:1295 (1964).

26. Howe, J.A., J. Chem. Phys. 39, 1362, 1963.

27. Scribner, B., Metallurgical Applications of Laser Probe Analysis, J. Am. Chem. Soc. Abstracts of 149th Meeting, Apr. 5-9, 1965.

Microscopy and Holography

The advantages of using monochromatic, coherent light to obtain improved contrast in interference fringes in microscopy was proposed by Townes (1). Barnes (2) discussed the possibility of developing a microscope which will allow one to do matched spatial filtering in a microscopic system. References given include that of Vander Lught (3-6). Experiments were performed by Barnes on phase microscopic systems, using a helium neon beam as a source. Previous studies by Fine and Klein (unpublished data), using 6328 Å illumination indicated that this wavelength appeared to be relatively unsatisfactory for direct visualization in microscopy probably because of the decreased sensitivity of the eye at this wavelength. However, with the increasing number of wavelengths available, possible improvements on direct viewing as well as in the photographic improvement in contrast discussed by Townes may be obtained.

Applications of Holography

The advent of laser beams possessing high temporal and spatial coherency has given increasing impetus to holography studies, originally described by Gabor in 1949 (7). The basis of this "lenseless photography" is the reproduction of a 3 dimensional image of the original object in two steps. The first stage is the formation of an interference pattern on a photographic film, due to the interference of reflected (or diffracted) rays from an object, illuminated by a coherent source with the reflected reference beam obtained from a mirror. In the second stage, when a coherent beam is passed through this

photographic film, and observed, an image of the scene similar to that viewed by binocular vision is observed.

Magnification can be measured in two ways. It can be obtained by geometrical magnification. A portion of a fly's wing magnified by geometrical techniques is shown by Stroke (10). A second method is by irradiation of the scene at one wavelength, and then irradiating the interference pattern on the film at a longer wavelength. In the studies by Gabor (7) a mercury vapor lamp was used as a source, and holograms obtained at these frequencies. Gabor intended to use the technique for improving the resolving power of electron microscopes. A diverging electron beam would be used to produce the diffraction pattern on the film. The film would then be viewed with visible light resulting in magnification. He suggested its application to X-ray microscopy. X-ray microscopy has not been feasible in practice because of the unavailability of practical focusing systems at these wavelengths. The systems proposed using holography were, however, incapable of resolving points less than 10,000 Å, as two fringe patterns of the two points are too closely spaced for the X-ray plate to resolve. (X-ray wavelengths are of the order of 1 Å.) Stroke and Falconer (8, 9) suggested that this could be overcome by deflecting all the waves diffracted by the object into a direction where they would make a zero (or very small angle) with the reference wave, and thus maintain separability of the various waves. Techniques for achieving this effect, and initial experiments are discussed by Stroke (10). The technique of holography applied in the X-ray region, may permit three dimensional visualization of macromolecules, such as myoglobin, to be achieved in much shorter time than heretofore possible.

An excellent, non-mathematical presentation of principles of holography is given by Leith and Upatnieks (11). Further studies on holography were discussed at the Optical Society of America 1965 Spring meeting (12-15). Since considerable energy density is required for sufficient exposure of the photographic plate, time periods of the order of minutes are required with present day HeNe lasers. Holography utilizing low power gas lasers are consequently of little value for motion studies. Holograms made with pulsed lasers (11), may prove of interest in biology.

References - Microscopy and Holography

1. Opt. Masers and Poss. Applications to Biol., C.H. Townes, Biophys. J. 2:325-329, Mar. 1962.

2. Frank Barnes - Personal Communications.

3. "Signal Detection by Complex Spatial Filtering", A. Vander Lught, I.E.E.E. Transactions on Information Theory, pp. 139-145, Apr. 1964.

4. "Optical Filters: Their Equivalence to and Differences from Electrical Network", T.P. Cheatham, Jr., and A. Kohlenberg, 1954 IRE Convention Record, pt. 4, pp. 6-12.

5. "Spatial Filtering in Optics", E. O'Neill, IRE Trans. on Information Theory, Vol. IT-2, pp. 56-65, June 1956.

6. "Optical Data Processing and Filtering Systems", L.J. Curtona, et al, IRE Trans. on Information Theory, Vol. IT-6, pp. 386-400, June 1960.

7. D. Gabor, Proc. Roy. Soc. (London) A 197, 454, 1949.

8. G.W. Stroke and D.G. Falconer, Phys. Letters 13: 306, 1964.

9. G.W. Stroke and D.G. Falconer, in Symposium on Optical and Electro-Optical Information Processing Technology (Nov. 9-10, 1964), J.T. Tippett et al (Eds.) (MIT Press, Cambridge, Mass., to be published).

10. Lensless Photography, G.W. Stroke, Internation Science and Technology, pp. 52-60, May 1965.

11. Photography by Laser, E.N. Leith and J. Upatnicks, Scientific American 212 (6): 24-35, June 1965.

12. Abstract # WB12. Optical Soc. of America, 1965 Spring Meeting, Some Effects of Coherence on the Wavefront Reconstruction Process, G.O. Reynolds.

13. Abstract # WB13 Optical Soc. of America, 1965 Spring Meeting, Hologram Microscopy and Lens Aberration Compensation by the Use of Holograms, E.N. Leith, J. Upatnieks, and A.V. Lugt.

14. Abstract # WB14 Optical Soc. of America, 1965 Spring Meeting, Magnification and Third-Order Aberrations in Holography, R.W. Meier.

15. Abstract # WB15 Optical Soc. of America, 1965 Spring Meeting, Attainment of High Resolutions in Wavefront Reconstruction Imaging--II., G.W. Stroke and D.G. Falconer.

Tissue Culture Studies

Most of the investigations on effects of laser radiation on tissue culture have been carried out by Rounds et al (1,2,3). These studies were conducted at 6943 Å, at 3471 Å by frequency doubling 6943 Å with an ammonium dihydrogen phosphate crystal and at 5300 Å by frequency doubling 10,600 Å. Effects of both non-Q-switched and Q-switched irradiation were investigated. Studies at 10,600 Å were not reported. Methods for determining energy and power require documentation, to the extent possible.

The importance of pigment and dyes is indicated by the following experiments. At 25 joules/cm^2, 1 millisecond pulse duration (at 6943 Å), tissue cultures of pigmented cells (Negro skin, pigmented rabbit retinal epithelium, pigmented mouse melanoma), were immediately destroyed, while unpigmented counterparts (skin from a Caucasian donor, retinal epithelium from an albino rabbit, unpigmented mouse fibroblastoid elements) showed no morphological changes for atleast 24 hours post irradiation (1). In a second report (2), studies at 1,000 joules/cm^2 resulted in immediate cellular death of pigmented cells, without evident injury to non pigmented cells at this energy level. Prior staining with a vital dye, Janus Green, increased the sensitivity of nonpigmented cells to laser injury. Chick retinal epithelium cells containing more than eight pigment granules were destroyed, while those with less than eight survived.

At 3471 Å, obtained by frequency doubling from a Q-switched laser, cytoplasmic blebbing within ten minutes, followed by death within seventy-two hours, occurred on exposure of unpigmented cells at 0.2 joules/cm^2. There was no gross evidence of injury on irradiation at 6943 Å at 0.8 joules/cm^2. In these studies, at 3471 Å, and 6943 Å, cellular absorption measurements are required to determine the relative absorptivity and consequently, the sensitivity of the cell at the two wavelengths, Since the energy absorption is heterogeneous throughout the cell, and probably differs in distribution as a function of wavelength, the problem is complex.

Chick retinal pigmented epithelium cells were destroyed at 5300 Å, frequency doubled from 10,600 Å. The energy and power levels associated with destruction of these cells would be of interest.

Excised hearts from 3-6 day chick embryos showed slowing of rate and irregularity of contraction following irradiation at 2.5 joules/cm^2 at 6943 Å (2). The contractile activity of all three types of muscle (cardiac, smooth and skeletal) was inhibited by laser irradiation (2). It would be of interest to note whether these effects are similar to, or differ from that produced by heat. The temperature gradient, particularly as a function of time, will be dependent upon the power levels at which the energy is supplied.

The DNA synthesis of Hela cells was slowed, the proplase duration of human adenocarcinoma cells delayed, and mitosis of salamander lung cells stopped without apparent chromosomal injury. The synergistic

effect on a line of human adenocarcinoma cells of laser radiation at 6943 Å in conjunction with cobald gamma irradiation was explored. Further studies will probably be required to determine whether the effects are synergistic. Should synergism be shown, it will have been shown only for the system studied.

Chromosomal alterations have been obtained. Dicentric chromosomes, chromatid breaks and shift of the modal value for a line of rabbit aortal endothelium from 42 chromosomes to 40 were produced. This indicates that genetic transformations may result from laser irradiation at 6943 Å.

Other studies were oriented towards determining the site and mode of interaction of laser radiation within the cell. Mitochondia were irradiated at 5300 Å (Q-switched) without significant alteration. Alteration may have resulted if more energy were used - the energy of the radiation consequently requires documentation. Irradiation of DPNH together with lactic dehydrogenase at 3471 Å caused a 60% reduction in DPNH formation, whereas irradiation of either DPNH or lactic dehydrogenase did not affect the subsequent reaction rate. The effect on reaction rate of combining separately irradiated DPNH and LDH was not reported. This may be of significance, as it would indicate whether separate irradiations of both would result in end products, which in themselves affect the reactions.

Inhibition of ATPase activity was proposed as a working hypothesis. The basis for this is presented but may required further documentation.

Although the author considers that the cytotoxic effect produced by laser radiation is wavelength specific, he probably means wavelength dependent. He is aware of the physical as well as the biological complexities of the system undergoing irradiation, and the necessity of further study to elucidate the parameters of importance. Studies on threshold determinations would be desirable. It would be of interest to compare thresholds with in vivo threshold studies on the eye such as those by Ham et al, at the various wavelengths, and to determine the relative effect of Q-switched radiation.

Effects due to irradiation with a Mercury arc lamp at 17.9 watts/cm^2 for 10 minutes were carried out and may be significant per se, but cannot be compared directly with effects of laser radiation, because of the total energy density (in excess of 10,000 joules) applied with the arc, the lower power level at which it is applied, and the wavelength band used.

Determination of the relative and absolute absorption of energy by the cells would be of significance, but is of course, difficult. This, in conjunction with studies at various energy levels would permit a determination of two factors (1) the relative sensitivity of pigmented and unpigmented cells to radiation at that wavelength and (2) the thresholds at which destruction occurs. The potential cytotoxic effect on unirradiated cells in the vicinity requires further consideration.

In summary, chromosomal abnormalities have been obtained. Mitosis of salamander lung cells has been stopped without apparent chromosomal injury. DNA synthesis has been slowed. Physiological processes, particularly of the heart and of the intestine have been affected. The importance of pigmentation has been studied.

Many of the extensive studies discussed were considered by the author as preliminary at the time of publication, or when presented in a progress report. It is probable that many of these studies will be further refined, threshold measurements made, and the effects compared with those found on in vivo studies. The author is aware of the in vivo studies being carried out, and correlates the results of his studies with in vivo effects observed. Further attention should be directed to radiation measurement, within the limits feasible.

Knowledge of the normal, unirradiated, development of the cell lines used, is of course, necessary in order to meaningfully assess the effects of laser radiation on tissue culture, which are being intensively and scientifically pursued by Dr. Rounds and his colleagues.

References

1. Rounds, D.E., Federation Proc., 24, supp. 14, (1), Pt.III, 1965.
2. Rounds, D.E., et al, Conf. Lasers, N.Y. Acad. Sci., 1964.
3. Rounds, D.E., et al, Boston Laser Conference, Aug. 1964.
4. Rounds, D.E., personal Communication.

STUDIES ON INTERACTIONS WITH MACROMOLECULAR BIOCHEMICAL PREPARATIONS

Interactions of in vitro biologic systems with radiation in the ultraviolet, visible, and infrared regions of the electromagnetic spectrum have been studied at low-power levels (1). The effects of pulsed irradiation on biological preparations were studied at relatively high energy density (of the order of 10 joules/cm^2). These studies employed primarily flash photolysis, utilizing the discharge of energy stored in a capacitor bank through a flash tube. The purpose of the flash photolysis experiments was to investigate free radical formation, absorption spectra of excited states, and products formed immediately after irradiation. Although most flash photolysis studies were carried out in the gaseous phase, studies on solutions of biochemical preparations have been reported by Gibson and Ainsworth (2) and Grossweiner and Mulac (3). In studies on pulsed photoexcitation of ovalbumin by Grossweiner (4) and by Grossweiner and Mulac (3), broad transient absorption spectra of both helium-saturated and air-saturated oval bumin were determined. The transient absorption spectra of smaller molecules were studied to elucidate the sites of photon interaction on the macromolecule.

The development of lasers made available sources with high-power densities as well as high energy densities at relatively narrow band widths as compared with those obtained from flashtube systems. The objectives of studies on the effects of laser radiation on macromolecular preparations of biologic origin include: 1. Determinations of changes in biological activities or in physical-chemical properties and elucidation of parameters affecting such alterations. 2. Exploration of effects of energy transfer agents on inducing or altering the interaction. 3. Investigation

of differential interactions with diverse biochemical preparations.

Initial studies were carried out by Klein, Fine, et. al. (5) to determine whether specific effects of biologic significance could be demonstrated after laser irradiation of in vitro biologic systems. The observations made in these exploratory studies provided a basis for further studies of the interactions of laser radiation with biologic systems.

Studies on the effects of laser radiation on macromolecular preparations of biological origin were concerned particularly with components of enzyme systems and immuno-chemical systems. Enzymatic or immunological reactions can be detected and characterized at considerably higher levels of sensitivity than usually attained by chemical or physical-chemical studies. Subtle changes in molecular conformation may thus be reflected by enzymatic and immunologic methods. The purpose of these studies was to determine whether laser radiation could produce functional or structural changes in macromolecular preparations and whether these could be demonstrated to bear a relationship to each other. While functional changes were demonstrable by enzymatic and immunchemical techniques in various macromolecular preparations following laser irradiation, chemical and physical-chemical techniques used so far have not been sensitive enough to indicate the specific associated structural changes. It has been possible, however, to demonstrate that the functional changes (and presumably, the structural changes) induced by laser radiation differ from those produced by conductive heat. This suggests that the interaction of laser radiation is not due to ordinary thermal factors alone. It further suggests that high intensity radiation such as available through lasers, may offer

an additional tool for studying properties of macromolecules.

Enzymatic Studies

One of the preparations chosen for these studies (5) was a lipase system, since the activity of this enzyme can be affected by several categories of macromolecules, including the lipase itself, the lipoproteins, and albumin or other proteins acting as fatty acid acceptors. The action of lipase, furthermore, can be assessed by analytical ultracentrifugation in terms of changes in the distribution of the lipoproteins which provides a sensitive means for studying some aspects of the functional as well as structural characteristics of these macromolecules.

Pancreatic lipase preparations in several steges of purification were studied. In the less purified form, this lipase preparation contains other enzymes such as proteolytic enzymes (trypsin, chymotrypsin),amylases, phosphatases, and various nucleases. These relatively impure preparations are suitable for assessing differential interaction of laser radiation with various enzymes present in the same preparation. Such differential interaction between the effects of laser radiation on lipase activity and proteolytic activity, respectively, was demonstrated.

The conditions of irradiation described below for the studies on lipase (5) were essentially the same as those used by the same authors and their associates for the investigation of other macromolecules. Such modifications as were made are indicated in the summaries of the respective studies.

Most of the studies reported were carried out with a ruby laser operating at a wavelength of 6,943 Å and energy levels ranging from 3 to 100 joules .

per pulse with a pulse duration of the order of 1 msec. Exploratory studies were carried out with pulsed laser units employing neodymium-doped glass, operating at a wavelength of 10,600 Å, at energy levels exceeding 800 joules per pulse. Radiation was unfocused or defocused by simple lenses to provide spot sizes of 8 to 14 mm. in diameter unless otherwise stated in the experimental section. These spot sizes were usually sufficient to be equal to or larger than the maximum cross section of the irradiated specimen.

Aliquots of macromolecular solutions or suspensions were usually irradiated in 7-mm. test tubes or cuvettes. The volumes ranged from 0.1 to 0.5 ml. per aliquot. When larger volumes were required, the samples were irradiated successively and pooled prior to determinations of biologic activities or structural characteristics. To some of the preparations a solution of methylene blue was added to provide final concentrations of the dye ranging from 0.1% to 0.0001%. Aliquots containing methylene blue were compared with aliquots of the same preparation to which the dye had not been added. Controls included nonirradiated specimens, with or without methylene blue, and specimens irradiated in the absence of methylene blue, to which the dye was added immediately following irradiation.

Lipase preparations were obtained from hog pancreas purified up to stage V of the method of Baskys et al. (6). The lyophilized preparation was suspended in a 0.15 M solution of sodium phosphate, pH 8.6, to give a concentration of 0.001% of the dye. Preparations containing methylene blue and control preparations were irradiated with pulses at

at energy levels ranging from 3 to 100 joules; the number of exposures per sample varied from 1 to 20 at intervals ranging from 5 to 10 min. between successive exposures. The radiation was delivered unfocused at a distance of 20 cm. from the face of the ruby.

After irradiation the lipase preparation was diluted with 9 volumes of buffer to a concentration of 2 mg. of lipase preparation per ml. of buffer; aliquots of the latter concentration were serially diluted to a level of 2 gamma of lipase preparation permilliliter of buffer.

The activity of the lipase at the various dilutions was tested for deturbidification and release of free fatty acids as previously described (6). The activity of the lipase decreased as the total energy of the radiation was increased in the presence of the dye, while in the absence of methylene blue changes in lipase activity were not significant.

Less highly purified lipase preparations which contained appreciable levels of proteolytic activity were irradiated to determine whether different effects would be produced on the respective enzyme activities. Proteolytic activity was not altered by laser radiation whether methylene blue was present or not, while the lipase activity was reduced or eliminated in the presence of the dye.

Lipase contains two essential components, each of which is inactive without the presence of the other, while recombination of the separate components results in reconstitution of the original level of activity. One of these components is labile when kept at 56°C for 10 minutes, or more, while the other component is stable to heat at 100° C. for an hour or longer. Following laser irradiation, it was found the lipase component which is stable to heat at 100° C had been de-activated, while the component labile at 56° C. did not lose activity. These observations suggest that

factors other than the usual thermal effects may be involved in the interactions of laser radiation with lipase preparations.

Studies on a number of enzyme preparations were reported by Igelman et. al. (7). The authors found that tyrosinase was not activated by radiation at 6,943 Å in vivo or in vitro, while the activity of this enzyme is known to be increased by X-rays or ultraviolet radiation. The other preparations which were studied (8) in vitro, included trypsin, lysozyme, catalase, peroxidase, alcohol dehydrogenase and serum amylase. Laser radiation at 6,943 Å and at 10,600 Å. was used. The energy levels of the ruby laser radiation was 45-85 joules per pulse in a non-Q switched mode, and 0.5 joules per pulse at a peak power level of 10 Mw Q switched. Neodymium radiation was non-Q switched at 9 joules per pulse. The spot sizes or the energy and power densities are not stated. From 1 to 7 exposures were employed, which persumably were successive, although the intervals between exposures are not stated.

The only effect noted was a 30-50% reduction in peroxidase activity, which is known to be photolabile. The other enzyme activities remained unaltered. No attempts were made to study the effects of laser radiation in the presence of dyes or other energy absorbing or transfer agents.

The authors conclude that "if laser radiation of the type used in these experiments and under these experimental conditions has a significant ionizing potential, it could be expected to affect all enzymes in a fashion similar to X-ray irradiation" They state, "that this is not the case as indicated by the results"(i.e. lack of effects),"and it appears that this laser radiation has no unsuspected property that is deleterious to enzymes in general."

Although the authors did not find alterations in specific enzymes under the conditions stated, their conculsions cannot be extrapolated to all enzymes, nor to laser radiation (at 6943 Å) at all energy and power density levels. At high peak power densities, ionization is produced in air. Free radical formation in biological systems including enzyme preparations has been demonstrated with a high degree of probability following laser irradiation (9). Charged particles have been produced (10) by laser irradiation of biological material. The production of hypersonic frequencies on irradiation of liquids may be of significance in aqueous enzyme preparations. Since the actions of ultraviolet radiation (which is ionizing) differs from that of X-irradiation, the effects should not have been compared to "ionizing" radiation in general.

The enzymatic activity of plasmin (or firinolysin) is demonstrable on a number of different substrates, such as fibrin, other proteins, (i.e. casein), plasminogen (or profibrinolysin) which is converted (activated) to plasmin (fibrinolysin), and tosyl argenine methyl ester (TAMe) or other synthetic substrates, in which an ester linkage is cleaved. The action on these substrates is referred to as fibrinolytic, caseinolytic, activator and esterase activities, respectively.

The various parameters of plasmin activity were investigated (5) following laser irradiation at 6943 Å in order to determine whether the activities of an enzyme preparation could be differentially altered by laser irradiation in respect to the diverse substrates upon which it acts. In addition to being a suitable preparation for these studies, the general effects of laser radiation on fibrinolysin were of interest because of the

increasing recognition of the physiologic significance and therapeutic applications of this enzyme system. One of the advances in hematology in recent years is the realization that the blood-clotting system is not alone responsible for maintaining hemostasis and hemofluidity, but that other systems such as the retractozyme system and the fibrinolysin system have to work in harmony with the clotting system to maintain a homeostatic balance. Since someof the studies on laser irradiation of fibrinolysin preparations bear on the complexity of this system, the current status of the fibrinolytic mechanism is briefly outlined.

It was suggested by several investigators (11) that the clotting of fibrinogen is a continuous process which is in dynamic balance with the continuous lysis of fibrin. As a result of this balance, a thin, possibly mono-molecular layer of fibrin is continuously present on the luminal aspect of the blood vessel wall. Hyperactivity of the fibrinolytic system may lead to hemorrhagic tendencies; overbalance of the clotting components and relative hypoactivity of the fibrinolysin mechanism may result in a thickened fibrin meshwork which may then tend to trap large molecules of lipoproteins, thus initiating the formation of atheromatous plaques (12). The fibrinolysin system is not only of physiologic importance, but more recently several of its members have entered the therapeutic armamentarium. Most human tissues contain activators which are able to convert plasminogen, a normal plasma component, to the active enzyme, plasmin. Activators of this system are also found in some bacteria and fungi. Plasmin is able to digest fibrin and other proteins, including such nonspecific proteins as casein. There are other normal plasma components, called anti-plasmins, which are able to form a complex with plasmin.

Fibrin can compete for plasmin with antiplasmins, absorbing plasmin to its surface. (13) This phenomenon explains the specificity of plasmin for fibrin and the fact that fibrin clots can be dissolved with doses of plasmin which will produce little change in other clotting factors and plasma proteins.

Astrup (14) in explaining the mechanism whereby SK (streptokinase), a bacterial enzyme, can activate plasminogen, suggested that another plasma protein call pro-activator is involved, which is changed by SK to an activator, which is then able to act on plasminogen. Human blood seems to contain a great deal of this pro-activator, while plasma of a number of mammals contains much less and some species, for example, cattle, seem to contain practically none.

A hypothesis has been developed on the significance of the fibrinolysin system in hemostasis and wound healing. When tissues are injured, thromboplastins are liberated, partly from the injured tissues and partly from the platelets which disintegrate on the rough surface of the wound; they will produce rapid clotting and thus cessation of hemorrhage. At the same time, tissue activators, which will activate plasminogen, are liberated from the tissues. Plasmin is then adsorbed to the fibrin clot as it is formed, while in turn any free plasmin will be neutralized by antiplasmins. Endothelial regeneration will commence, and soon the continuity of the blood vessels will be re-established. The blood clot will become intravascular and is responsible for preventing the re-establishment of the circulation. In the meantime, however, the slow fibrinolytic effect of plasmin will result in recanalization and possibly the complete elimination of the clot. Teleologically the rapid action of the clotting system and slow action of the fibrinolysin system is readily understandable.

In addition to its fibrinolytic and caseinolytic activity, plasmin also has an esteratic effect and is able to split such synthetic esters as TAMe (tosyl-arginine methylester). If SK is added to human plasminogen, maximal fibrinolytic and caseinolytic activity develops very rapidly. As time passes, the enzyme acquires an esteratic activity as well (15).

If increasing amounts of SK are added to a certain amount of plasminogen, the fibrinolytic activity first increases until a maximum is obtained, and further amounts of SK will then be inhibitory. The same phenomenon is observed as far as the caseinolytic effect of plasmin is concerned: Namely, with the increase in SK concentration we get first increased and then decreased activity. However, if plasmin is measured by its TAMe esteratic activity, the inhibiting effect of high SK concentrations does not manifest itself.

Plasmin is not considered to be a single enzyme; there are several molecular species of plasmin which possibly transform continuously into each other. At least three related enzymes - alpha, beta, and gamma plasmin - have been characterized (16). This may be an analogous situation to the activation of chymotrypsinogen into chymotrypsin through a series of molecular forms.

Aliquots of a 10% solution of plasmin in distilled water were exposed to laser radiation in the presence and absence of methylene blue (final concentration of 0.05%). The energy per pulse varied from 32 to 72 joules, and from 1 to 75 successive pulses were delivered at intervals of 5-10 min as unfocussed radiation at 6943 Å with a pulse duration of the order of 1 msec. The fibrinolytic activity, the plasminogen

activator activity, the caseinolytic activity and the TAMe esterase activity were determined as previously described (17,18,19).

In the absence of methylene blue the above parameters of plasmin activity remained unchanged after laser irradiation. In the presence of methylene blue the activities of the plasmin preparations were decreased (5,20). After 40 successive exposures at 50 joules per pulse, fibrinolytic activity was reduced to insignificant levels, while appreciable activities were apparent in determinations of plasminogen activator activity, caseinolytic activity, and TAMe esterase activity.

To determine whether these phenomena reflected the effect on the plasminogen molecule or extended to the activator, urokinase was irradiated in parallel studies by Ambrus et al. (in preparation). In the presence of methylene blue as the energy transfer agent, the activity of urokinase in the activation of plasminogen was markedly reduced by laser irradiation.

In parallel studies (9), it was observed that laser irradiation of plasmin preparations was followed by the appearance of free radicals, while radiation of the same quality did not produce free radicals in a number of other enzyme preparations. The relation between alteration of enzyme activity and free radical formation after laser irradiation has not been investigated.

Calf thymus DNA (125 µg/ml) in the presence of methylene blue (0.125 µg/ml) was exposed to laser radiation at 6,943 Å under the conditions described above for studies on lipase preparations. Approximately 20% reduction of priming activity in a regenerating rat liver-DNA-polymerase system (21) was observed. No reduction was found in the absence of methylene blue. These were preliminary findings and require further study.

Studies on blood group substances

Studies on blood group substances were carried out in order to explore whether laser irradiation would alter the specific reaction of an antigen with a corresponding antibody (5). The activity of the blood group substances was assayed by inhibition of hemo-agglutination (22). Commercial blood group substances A and B were diluted to 1:8 or 1:32 with 0.85% saline buffered to pH 7.2 with M/150 phosphate. The diluted substance was divided into 2 aliquots, one of which was exposed to laser radiation. The conditions of irradiation were as described above for studies on lipase preparations, except that dyes were not added; the other aliquot was retained at 4°C. as a control.

The degree of inhibition of hemagglutination was determined by incubating 0.5 ml. of blood group solution with 0.5 ml. of commercial anti-A or anti-B (dilution 1:16) for 1 hour at 25° C. The control for this consisted of 0.5 ml. of buffered saline incubated with 0.5 ml. of dilute antiserum for 1 hour at 25° C. After incubation, the mixtures were serially diluted in 10 x 75 mm. test tubes. To each tube 0.5 ml. of 2% fresh group A or B erythrocytes were added. Tubes were shaken and the cell-polysacchride mixture incubated for 1 hour at 25° C. The tubes were centrifuged for 1 minute at 1,000 RPM in an International Model Clinical Centrifuge. Agglutination was read macroscopically after shaking tubes 5 times manually. The degree of inhibition was manifested by reduction of hemagglutination titers and/or the agglutination scores from those of the controls.

Following laser irradiation, the activities of the blood group substances were found to be increased. The activity of blood group substance B

was more markedly affected, than that of blood group substance A. Heating of preparations of the blood group substances at 100° C. for 10 minutes did not alter their activities. The inhibitory activity of irradiated (and control) specimens in the hemo-agglutination test was not significantly altered by dialysis. The electrophoretic mobilities and the boundaries observed on analytic ultracentrifugation did not reveal differences between irradiated and control specimens.

The irradiation of blood group substances which resulted in an increase in immuno-chemical activity suggests that the number of functional groups required for the specific interaction of agglutinogen with iso-agglutinin may have been increased. This could have occurred due to fragmentation of the molecule resulting in the liberation of active groups. Alternately, the molecular conformation of the blood group substances may have been altered in such a manner that previously inaccessible functional groups were relocated into positions in which they were available for reaction. Interaction with laser radiation may also have produced functional groups de novo or destroyed an inhibitory activity. Since dialysis of the irradiated specimens did not alter their activities, relatively large molecules appear to be associated with the increase in the agglutinogen activity. Failure to reveal differences between the electrophoretic and ultracentrifugal patterns of the controls and of the irradiated preparations does not exclude involvement of molecules, which may have been too large to be dialyzable, but not large enough to be reflected by changes in the moving boundaries.

Studies on gamma globulin

Studies on gamma globulins were carried out to investigate the effects of laser radiation on proteins in regard to protein-protein interactions (5). Serial dilutions of the laser treated, heat-treated, or control HGG were prepared in 0.85% saline containing 1:10000 parts of Merthiolate (Eli Lilly). The initial dilution was 1:125, based on the total concentration of protein of the particular serum. Activity of gamma globulin was determined by several methods. The percipitin test was carried out by placing 2.7 ml. of each dilution of serum in a haemoscope curvette and recording the photoelectric reading of turbidity. To each curvette was added 0.4 ml. of rabbit anti-HGG serum. Each curvette, as well as the control were incubated at 37.5°C. Readings were made photoelectrically at 20, 40 and 60 minutes, respectively. Correction was made for turbidity contributed by dilutions of globulin alone, as well as by the rabbit anti-serum alone. Summation of points of the precipitin curve were proportional and representative of the curve areas of the precipitin reactions. The peak of each curve occurred at the optimal proportions (23) of antigen and antibody. To the left of the peak was antigen excess, to the right antibody excess. It was possible to evaluate shifts to left or right as demonstrations of effect of irradiation, or heat treatment upon HGG antigen.

Laser treated heat-treated and control HGG preparations were studied by immunodiffusion. These preparations were added to 0.3 ml. capacity wells molded into 1% Difco Agar in saline. The reactions wells were located in a circle surrounding a 0.3 ml. capacity well containing rabbit

anti-HGG. The formation, number, and identity of precipitin arcs was noted at 16, 24, 48, and 72 hours. Photographic record and analysis of precipitin arcs followed each experiment.

The microimmunoelectrophoresis technique of Scheidegger (24) was employed on standard microscope slides, using Agafor (National Instrument Company) equipment. A veronal buffer (pH 8.6) was used in the buffer compartments. Power used was 6 volts per cm. Separation was done at 45 minutes. The goat anti-human serum (I.E.P. Hyland) was placed in a center trough on each diffusion slide after electrophoretic separation of HGG in the micro-reaction wells, that bracket the trough. After 16 hours of incubation in a moist diffusion chamber, the slides were visually inspected. Slides were dried and the protein arcs stained with light green SF dye. Position, shape, and density of protein arcs were analyzed macroscopically.

In the reaction of antiserum with gamma globulin, maximum precipitation occurred at lower concentrations of antigen (i.e. human gamma globulin) when it had been irradiated, then with the untreated controls. The curves for the reactions at serial dilutions of the irradiated specimens at constant levels of antibody were shifted to the right of the control curves. The irradiated gamma globulin preparations reacted less actively with rheumatoid serum than the untreated controls while heated gamma globulin showed a more intensive reaction. On gel diffusion and immuno-electrophoresis there were no apparent differences between irradiated and control gamma globulin precipitation arcs.

Discussion

The data presented suggest that interactions of relatively coherent, monochromatic electromagnetic radiation at high-peak power with biologic systems in vitro can result in changes of some of the properties of these systems. The data are preliminary in that only some qualitative aspects of the biologic properties of macromolecular preparations were explored. The desirability of further quantitation of parameters of the radiation, of the interactions, and of the effects on biologic activities is indicated by these initial explorations of the availability of suitable systems and the feasibility of the requisite studies.

In the studies on partially purified lipase preparations, a reduction in the rate of lipolysis was evident. The initial rates of enzymatic activity were considerably reduced following irradiation in the presence of methylene blue, as compared with the non-irradiated controls, whether the latter contained methylene blue or not. The addition of methylene blue resulted in increased absorption of the incident radiation. At pulses of 35 joules, approximately 14 joules were transmitted in the absence of methylene blue, and no transmission of energy was detected in the presence of the dye. Therefore, a maximum of 35 joules could have been absorbed, less the energy reflected or scattered by the system under these experimental conditions. Irradiation at higher energy levels, at which the difference between incident and transmitted energy was greater than 35 joules in the absence of methylene blue, did not result in reduction in rate of lipolytic activity. This would suggest that methylene blue acts as an energy transfer agent, which may sensitize the lipase system to the

absorbed energy in addition to increasing the amount of energy absorbed. Another possibility is that the resultants of the interaction of methylene blue with high-peak-power radiation at 6,943 Å are different from those of the components of the lipase preparation.

The lack of overt effects on the proteolytic activity, present as an impurity in the lipase preparation, at energy levels at which irradiation reduces the rate of lipase activity indicates differential effects on different enzymes. It has previously been observed (6) that the activity of pancreatic lipase is heat labile (100° C. for 5 min.), while the proteolytic activity (trypsin, chymotrypsin) is not reduced under those conditions. The amount of heat that would be produced by the radiation, even if all the energy had been converted to heat (assuming uniform temperatures, i.e., 8° C., throughout the aqueous enzyme preparation) would not have been sufficient to reduce lipase activity to the same degree as brought about by laser irradiation. Transient temperature elevations, however, may have occurred without being demonstrable under the conditions of the experiments. These considerations, as well as observations made previously (25,26) and concurrently (20), suggest the possibility that other than the usual thermal effects may be involved in producing changes in the biologic activities of macromolecular preparations.

Since the lipase preparations were not pure, some of the effects observed may have been due to changes in one or more of the impurities. Thus contaminating proteins, which act as acceptor proteins for the triglycerides or free fatty acids, may have been altered with resultant reduction of their capacity for lipid acceptance. Alternately one or more of the impurities

may have been converted into inhibitors of lipase activity; such changes would then be indirectly responsible for reduction of lipolytic activity.

The observations on plasmin indicate that laser radiation may differentially affect the activities of an enzyme on its different substrates. This suggests that different activities of the same enzyme may be affected to different degrees or in different ways by electromagnetic radiation at relaltively high energy and power density. These effects may be due to differential primary interactions with the respective functional groups on the enzyme molecule, or to differential secondary effects of the same primary interaction. It may also be related to the heterogeneity of the enzyme preparation, laser radiation acting more effectively on some members of a population of closely related enzymes.

The initial rates of plasmin activation were considerably lower than those of the corresponding control preparations. The rate of the second phase of activation, which represents a relative decrease in fibrinolytic activity, however, was not altered by laser irradiation. This may further indicate a differential effect on enzyme activity at stages at which it is the limiting factor in the rate of the reaction, as compared with stages at which other rate-limiting factors predominate.

Since the plasmin preparations studied were not pure substances, it is possible that one or more of the activities were associated with components other than the fibrinolytic enzymes. Thus, laser radiation may have resulted in differential interaction with different molecules rather than with different functional moieties on the enzyme molecule. The interaction may also have resulted in changes in configuration of molecular

structure, altering the intramolecular spatial relations rather than altering the structures of the active sites on the enzyme.

The changes in the immunochemical properties of the blood group substances and of the gamma globulin preparations were produced at 6,943 Å without the addition of an energy transfer agent such as methylene blue. At the low-power levels of standard spectrophotometers, radiation at 6,943 Å is transmitted without any detectable absorption by these preparations. At the relatively higher power levels (10-100 kw. range) of laser radiation, absorption of energy at 6,943 Å occurs. The fraction of transmitted energy does not appear to be a linear function of the energy of the incident radiation. Thus considerable additional work is needed to determine the relationship between incident and absorbed energy at a given wavelength for these macromolecular preparations. Since it appears that the proportions of transmitted energy vary with different macromolecular preparations, it would be interesting to determine whether absorption of electromagnetic radiation at high-peak powers can be correlated with structural characteristics of the molecule. This may provide further indications of the relation of structure to function, in addition to the information that may be obtained from the effects of selective interaction with functional groups or the effects of conformational changes on biologic properties.

The non-linearity of the relation of transmitted to incident radiation was further indicated by solutions of methylene blue and other dyes. Standard spectrophotometric determinations showed various levels of transmission by the solutions of the dyes in the spectral region of 6,943 Å. The same solutions showed considerably lower proportions of transmitted

energy when the incident radiation was at a power level in the 10 to 100 kw. range.

Irradiation of preparations of human gamma globulin indicated differential effects on members of this molecular species. Irradiation resulted in loss of reactivity with serum of patients with rheumatoid arthritis, while energy of the same order of magnitude, applied by raising the temperature, did not increase the reactivity of the gamma globulin preparation in this system. In the reaction with heterologous antibodies to human gamma globulin, the irradiated antigen showed maximum reactivity at lower concentrations (of the antigen) than the untreated controls. Some of the irradiated specimens not only reacted maximally at lower concentrations, but also showed higher levels of maximum reactivity than the controls. Irradiation of heterologous antibodies to limulus serum appeared to increase the area of the precipitin curve, quantitated photoelectrically, in comparison with control specimens. Further studies are needed to elucidate the nature of this change. These observations further suggest that the interaction of laser radiation with these biologic systems was not entirely due to the usual thermal factors.

Interaction of the radiation with aqueous systems of macromolecules may primarily involve solute (or suspensoid) molecules rather than the water molecules. Similar considerations may pertain to dyes in aqueous solution. Thus, thermal effects at high intensities may be relatively sharply localized to the interacting molecules in an aqueous system. The usual reactions to generalized heating of the entire system may, therefore,

differ from the reactions to molecularly localized interactions.

Although the actual energy was not measured in these studies, relative absorption of energy was estimated on the basis of calorimetric measurements with and without interposition of the sample. Consideration of some of the physical factors involved in the interaction is suggested below.

The energy per quantum at 6,943 Å is about 2 ev., equivalent to 3.2×10^{-12} ergs., equivalent to 3.2×10^{-19} joules. If every molecule in a gram molecule absorbed a photon at 6,943 Å, the total amount of energy absorbed would be of the order of $3.2 \times 10^{-19} \times 6.0 \times 10^{23}$ joules per gram molecular weight or 1.9×10^{5} joules, which would approximate 40,000 cal. per gram molecule.

Since a 1-joule pulse at 6,943 Å contains 3.1×10^{18} quanta, a 100-joule pulse at 6,943 Å would contain 3.1×10^{20} quanta. The macromolecules under consideration have molecular weights of the order of 10^{5}. A solution containing 10 mg/ml will, therefore, represent an order of 10^{-4} M, and consequently contain of the order of 6.0×10^{16} molecules/ml. If the quantum efficiency approached 1, then all the atoms would be excited by a 100-joule pulse at 6,943 Å.

If the quantum efficiency of the interaction is 0.0001, (0.01%) for a specific reaction, then the per cent of molecules altered will be of the order of $(3.1 \times 10^{20} \times 10^{-4})/(6 \times 10^{16}) \times 100\%$ or 50% assuming single photon single molecule interaction. If the lifetime of the excited state was of the same order of magnitude as the duration of the pulse, then interaction between excited molecules could be considerable. Even if

the lifetime of the excited state is several orders of magnitude less than that of the pulse duration, a considerable number of molecules will still be in the excited state at any one time and interactions, normally not detected within the limits of chemical determination, may become apparent and measurable by endproduct analysis. Molecular changes due to interactions between excited states may consequently be obtained beyond those that could be recognized if the equivalent energy were delivered at low-power levels. Because of possible transient absorption spectra, the possibility of interaction of quanta with excited states of the atom also exist and may result in a detectable number of altered molecules not otherwise measurable.

References

1. Giese, A.C. (editor) Photophysiology, (New York: Academic, 1964.)

2. Gibson, Q.H. and S. Ainsworth, Photosensitivity of Haem Compounds, Nature 180: 1416, 1957.

3. Grossweiner, L.D. and W. A. Mulac. Primary Processes in the Flash Photolysis of Ovalbumin and Constituents, Radiation Res. 10: 515, 1959.

4. Grossweiner, L.D., Metastable States of Photoexcited Ovalbumin and Constituents, J. Chem. Phys. 24: 1255, 1956.

5. E. Klein, S. Fine, J. Ambrus, E. Cohen, E. Neter, C. Ambrus, T. Bardos, and R. Lyman, Interaction of laser radiation withbiologic systems. III. Studies on biologic systems in vitro, Fed. Proc. 24 (1 pt.3) suppl. 14: S104 - S 110, 1965.

6. Baskys, B., and E. Klein, and W. F. Lever. Lipases of Blood and Tissues. III. Purification and Properties of Pancreatic Lipase. Arch. Biochem. Biophys. 102: 201, 1963.

7. Igelman, J.M. and T. Rotte, Effects of laser radiation on Tyrosinase, Fed. Proc. 24 (1 Pt. 3) Suppl.14: S 94-S 96, 1965.

8. Igelman, J. M., T.C. Rotte, E. Schecter, and D. J. Blaney, Exposure of Enzyme to Laser Radiation, Ann. N.Y. Acad. Sci 122: 790-801, 1965.

9. Derr, V., E. Klein and S. Fine, Free radical occurrence in some laser-irradiated biological materials, Fed. Proc. 24 (1.pt. 3) suppl. 14: S 99 - S 104. 1965.

10. Fine, S., W. Norak, W. Hansen, K. Hergenrother, R. E. Scott, J. Donoghue and E. Klein, Measurements and Hazards on Interaction of Laser Radiation on Biological Systems, NERM Record 6: 158-159, 1964.

11. Ambrus, C. M. Clot Formation and Dissolution: Basic Considerations, N.Y. State J. Med. 62: 3738-3744, 1962.

12. Duguid, J.B.: Thrombosis as a factor in the pathogenesis of coronary Atherosclerosis, J. Path & Bact. 58: 207 (1946).

13. Ambrus, C.M. and Markus, G: Plasmin-antiplasmin complex as a reservoir of fibrinolytic enzyme, Am. J. Physiol. 199: 391 (Sept.) 1960.

14. Astrup,T: Fibrinolysis in the organism, Blood 11: 781 (1956).

15. Markus, G. and Ambrus, C.M.: On the formation of different types of plasmin by streptokinase activation, J. Biol. Chem 235: 1673-1960.

16. Markus, G. and Ambrus, C.M. Characterization of enzymes resulting from plasminogen activation. Fed. Proc., 24: 98, 1959.

17. Ambrus, J.L., C.M. Ambrus, N. Back, J.E. Sokal, and G.L. Collins. Clinical and experimental studies on fibrinolytic enzymes. N.Y. Acad. Sci. 68:97, 1957

18. Ambrus, J.L., C.M. Ambrus, J.E. Sokal, G. Markus, N. Back, L. Stutzman, D. Razio, C.A. Ross, D.H. Smith, A.C. Rakati, G.L. Collins, D.L. Kline and J.B. Bishman. J. Cardiol. 6: 462 -475.

19. Ambrus, J.L., et al:, Clinical and experimental studies with fibrinolytic enzymes. A progress report, In: Anticoagulatns and Fibrinolysins. (Philadelphia: Lea & Febiger, 1961) p.413.

20. Fine, S. and E. Klein, Biological Effects of Laser Radiation, Advan. Biol. Med. Phys. 1965 (in press).

21. Fine, S., E. Klein and Y. Laor, Modifications of Effects of Laser Radiation by Light Absorbing Chemicals, Boston Laser Conf. 3rd, Boston, Mass., 1964.

22. Klien, E. and S. Fine, The Biological Aspects of Laser Radiation, Absts. 149th Meeting Am. Chem. Soc1, Detroit, Michigan, April 1965. p 41.

23. Cohen, E., E. Neter, and B.M. Norcorss. Detection rhumatiod factors by means of photoelectric measurement without hemaglutination. Am. M. Clin. Path. 31: 507, 1959.

24. Scheidegger, J.J., Une micro-methode de l'ummuno-electrophorese, Int. Arch. Allergy 7: 103-110, 1955.

25. Fine, S., E. Klein, R. Scott, Laser Irradiation of Biological Systems, I.E.E.E. Spectrum 1: 31, 1964.

26. Fine, S., E. Klein, J. Ambrus, E. Cohen, C. Ambrus, V.E. Derr & W. Nowak, Interaction of relatively coherent laser radiation and biological systems. Fed. Proc. 23: 442, 1964.

THE LASER MICROBEAM

VI. THE LASER MICROBEAM

The concept of combining a source of radiation with a microscope to produce a microbeam to injure or destroy microscopic structures was first reported by Tchakhotine in 1912 (1). Following the active leadership of Tchakhotine a number of investigators have explored the effects of partial cell irradiation on cellular physiology. This work has been done on various biological systems using ultra-violet, soft X-ray, and high energy particle microbeams with beam diameters down to 1 μ. The small beam diameter was obtained with a wide variety of optics, including magnetic focusing of electrons, conventional, and reflecting microscope optics (2, 3).

When lasers became available one of the first applications considered for the laser was microsurgery (4). This was suggested because in theory the beam emitted by a laser could be coherent and very directional, having an angle of divergence approaching the theoretical diffraction limit, λ/D, where λ is the wavelength and D is the diameter of the end plate of the laser. Such directional coherent energy can be focused to an extremely small spot. The spot size obtainable with the coherent beam is limited only by diffraction. To a good approximation, this limit is $\lambda/2$, or for a ruby laser with a wavelength of 6943 $\mathring{A}$ the theoretical limit is approximately 1/3 micron (4).

In practice, this limit is very difficult to achieve, as lasers are not completely coherent and the area to be irradiated must be located in the exact focal plane of the lens system to achieve minimum spot size. Spot sizes of 1 μ diameter have been reported (5). A more complete discussion of the optical principles involved in a laser-micro-

scope system can be found inpapers by Malt (5)and by Peppers (6)and Fine et al (25).

The advantage of a laser microbeam system is the very high energy density which can be obtained over a small region. The power density at the focal plane of a laser-microscope system comprised of a ruby laser with 100 mj. output with a pulse duration of 0.5 milliseconds, focused through a microscope to a spot 2.5 microns in diameter would be approximately $4 \times 10^9 w/cm^2$. The power density for this same system focused to a spot 25 u in diameter would be approximately $4 \times 10^7 w/cm^2$. Townes (4) points out that a power density of $10^5 w/cm^2$ would have an electric field strength at the focus of about 10^6 volts/m. He indicates that ionization can be produced at these power levels at the focus of a laser beam. A laser microbeam is comparable in some respects to other types of microbeams, but differs because of the possible high electrical field strength. However, the field strength considered is that in vacuum and not the field strength in the target.. For example, for a perfect conductor the field strength within the conductor will be zero. Also, the configuration of the time varying field, even in vacuum, is extremely complex, and will not be discussed further at this point.

Instrumentation

Bessis and Nomarski, working in Paris, had developed an efficient uv microbeam system (7) which they altered to accommodate a ruby (8,9,10) or neodymium (11) laser rod. The microscope (fig. 1) used embodies a vertical illuminator. The object is placed on a dielectric mirror (M). The alignment is achieved by observing a second image of the object by autocollimation on the exit surface (m) of the laser (L), the brightness of which is controlled by two polarizers (P_1) and (P_2) and by a quarter

wave plate (Q) that is withdrawn during operation. It is then possible to localize the spot for the alignment of the apparatus by means of a very weak image resulting from the reflection on the anterior face of the laser. The laser used by Bessis employs a laser rod only 3 mm. in diameter and 50 mm. long; thus the beam produced is smaller in diameter than the pupil of the objectives of the microscope. This enables the whole microscope to be used to advantage, and the laser is placed in front of the eye piece. The eye ring is the only factor limiting the beam under these circumstances. The diameter of the eye ring used by Bessis is d_0 = 500/6 (N.D.) combined with a 6X ocular and a 100X objective. This system diaphragms the laser beam by 1/3 to give a calculated spot size of 2.5 μ. Another advantage of Bessis' system is the use of closed circuit TV to observe the specimen while it is being irradiated. A 16 mm. motion picture camera is also used to provide a permanent, dynamic record of the experiment, although the camera tube is blocked when the laser is fired. This system has incorporated in it precautionary measures to protect the investigators from the hazards of laser radiation. The oculars used for focusing are equipped with filters to protect the experimenter in case of accidental firing of the laser. A similar filter is placed in front of the TV camera to protect the vidicon tube from damage. Damage to the phosphorescent coating of the vidicon tube has been encountered by other investigators using closed circuit TV in laser research (12).

The laser microbeam system described by Bessis (8,10) stands as the most complete system yet to be reported in the literature. Unfortunately, there is no mention of the energy levels or energy densities produced by this system; consequently, it is difficult to compare the work done in Bessis' laboratory with results obtained by workers in other laboratories.

Another, much simpler, system which has been used by several different groups (15, 13, 14) has been developed on a commercial basis by T.R.G. This system consists of a triocular Leitz Ortholux microscope combined with a ruby laser mounted vertically so the laser beam is aligned with the optical path of the microscope. The laser uses a ruby crystal 6.25 mm. in diameter and 37.5 mm. in length. Maximum output of this laser is a nominal 100 m. joules per pulse, and the duration is 10^{-4} seconds. Using various combinations of oculars and objectives, Saks has been able to obtain beam diameters from 500 μ to 4.3 μ (16). Others using the TRG. system have reported beam diameters as small as 1 μ (5).

In a recent paper (6), N.A. Peppers of Optics Technology, Inc. discussed some of the theoretical and practical aspects to be considered in the designing of a laser microbeam system for bio-optical research. One of the practical aspects considered was the damage threshold of the objective. Damage thresholds of some optical cements for a 200 u sec ruby laser is about 1 j/cm^2. In this case, a cemented element objective with a 3 mm^2 cross section would be damaged by a pulse of only 30 mj. Therefore, with very powerful high energy laser beams, a good quality f/1 simple thin lens should be used to obtain a spot of about 25 microns diameter. On the other hand, if a radiation spot of a few microns or less is required, a very intense spot can be obtained with a compound microscope and a low energy laser. A typical laser beam of 5 m rad. divergence can be focused by a 10X eyepiece to a spot diameter of about 125 microns at the focal plane of the objective. A

100X objective with a high numerical aperture could further reduce the spot to approximately 2 μ. Such a system with its energy restricted to 30 mj would give an energy density at the primary focus (E_p) of approximately $10^6 j/cm^2$. The theoretical diffraction limit can be essentially realized in such a system, if one places an auxiliary diaphragm in the focal plane of the eyepiece, such that the relationship $D \ll Md_{min}$ is observed; where D = size of aperture, M = working magnification of the objective, d_{min} = theoretical diffraction limit. However, in this case the aperture reduces the spot size and E_p to relatively small values. Alternatively, if D is selected such that $D \gg Md_{min}$, the diffraction effects are negligible, and both the spot size and E_p are considered by the authors as being large. It is however probable that the energy density in this case will be greater than when $D \ll Md_{min}$. However, the actual energy density may not be large with the same laser. The author apparently does recognize this fact, since he states that a compromise must be achieved for which relatively high energy densities can be achieved for relatively small spot sizes.

With these principles in mind, a laser microbeam system was constructed employing a pulsed ruby laser mounted on a diffraction limited microscope. The laser uses a ruby crystal 6.35 mm. x 76.2 mm. and has a pulse duration of 0.2 milliseconds and a maximum energy output of 0.5 joules per pulse. The microscope is an ordinary monocular microscope with a 15 layer dielectric limiting aperture at the secondary focus of the objective. A variable iris diaphragm is placed in the focal plane of the eyepiece as an adjustable aperture to eliminate unnecessary radiation from being focused on the limiting diclectric aper-

ture. This is accomplished by adjusting the diaphragm for each objective used, such that the back aperture of the objective is just fully illuminated by the laser beam. This insures realization of the full resolving power of the objective, while preventing unnecessary radiation from damaging the dielectric aperture or the objective. For most of the experiments reported by Peppers, a 10X eyepiece was combined with a 50 μ limiting aperture and a 40X objective (N.A. = 0.65) to obtain a spot size approximately 2 μ in diameter. This system only allows about 2.8% of the original laser beam energy to pass completely through the system (first aperture with nominal diameter of 1 mm. passes approximately 4% of energy, objectives are assumed to transmit 70% of incident energy). However, on the basis of the damage to samples, the energy density of the laser microbeam was estimated to be of the order of $10^2 j/mm^2$. This is sufficient to hemolyze red blood cells, and to puncture cell membranes of various other tissues.

Attempts to combine a laser with a microscope were carried out by Malt (5, 17) where both a cw gas laser and a pulsed ruby laser were focused through one ocular of a binocular microscope. The second ocular was used for focusing. Lang, et al (18) also tried this system; apparently this arrangement is not very satisfactory, as both have abandoned it. Various investigators, in their studies, hoped to take advantage of the high degree of coherence, that can be obtained with a cw 6328 Å laser in order to obtain a spot size essentially equal to the diffraction limit ($\lambda/2 \simeq 1/3\ \mu$). Unfortunately, the relatively low power output of available cw gas lasers was insufficient to produce any discernable changes on the various biological samples tested (5, 17, 18).

Tomberg (19, 20) used a system consisting of a laser mounted vertically on top of a microscope tube, to which are connected two additional side tubes, one containing a viewing ocular, the other a dosimeter. After focusing is completed, a beam splitter in the main tube is rotated, so that an undefined part of the energy is monitored by the dosimeter. The exit energy, however, is not monitored. The results obtained with this system, such as crater production in a specimen and clotting of blood components are attributed to "mechanical effects similar to that observed on paddle wheels of radiometers", or high electric fields. No histological data is presented. No information is given as to why the "coagulation" results are due to high electric fields. The usual clotting factors are not discussed.

Complex, high electric fields can be produced in vacuo at the focus of a laser beam, but may not be significant within tissue, particularly since scattering is marked, and the attenuation constant may be high. The complexity of the coagulation system precludes its use for testing this hypothesis. Insofar as kinetic effects due to light, radiometer paddle wheels are not turned by radiation pressure, which in general is low. The hypotheses presented, therefore, bear no relationship to the experimental results obtained.

A laser microbeam system employing a ruby laser and a closed circuit TV system, available in Europe, was described by Locquin (21).

In 1965 Ramon Szpur, of Spacerays, Inc., developed a focusing system for use with either pulsed or cw lasers, based on reflection rather than refraction. Two highly reflective mirrors to focus the beam instead of 2 systems of lenses are used (22). Two specially formulated stainless steel mirrors provide an average transfer efficiency exceeding 80%, according to the manufacturer. The laser beam strikes a conical primary mirror and is reflected onto 2 parabolic secondary mirrors which focuses the radiation to a 100 μ diameter spot. This system has an advantage over a

refracting system in that it is not limited to the low energy or power levels that are below the damage threshold of refractive materials, According to the company that tested the instrument (23), it is also relatively free from chromatic aberrations which are common to reflective focusing systems.

All of these laser microbeam systems have one common feature, that is, they were designed and developed for use in biological research. Another interesting observation is that 5 of the 8 systems mentioned were developed by commercial corporations. This is some indication of the interest which has been shown in laser microbeam research in the biological sciences.

Applications:

With the advent of the laser, microbeam techniques, originally conceived as a tool for the studying of cellular physiology by partial cell irradiation (1), have become of interest to researchers in many different areas of Biology and Medicine.

Probably the simplest system used in what might be considered as a laser microbeam study, was reported by Witt, Reed and Tittel (24). They used a pulsed ruby laser, output 1 joule per pulse and pulse width of 1 millisecond, focused through a 32 mm. Bausch and Lomb microscope objective to obtain a spot 100 microns in diameter with an estimated power density of $10^7 W/cm^2$. This was used to irradiate 20 adult female spiders. The behavior of the irradiated spiders was then followed by periodically photographing and measuring the webs constructed by these spiders and comparing the webs constructed after irradiation with those constructed before irradiation and with the webs

of 13 controls. Histologic sections were made of the irradiated area of dead spiders, and the lesions examined microscopically. The spiders were followed for periods of 1 day to 6.5 months, the mean follow-up time being 65 days. Five of the irradiated spiders died within one week, one had leakage of fluid and the others had lesions of the oesophagus and the heart. The laser lesions were similar in general histologic appearance to those reported in mammals (25, 14) with the signal feature being the clear delineation of the lesion from the surrounding healthy tissue. The data on web construction following laser radiation was summarized in the following table (14).

				Permanent disturbance			
Target	Death	No disturbance of webs	Temporary disturbance of webs	Area of length only	Angles only	Spiral only	Multiple disturbances
Abdomen (N=4)	2	0	2	0	0	0	0
Cephalothorax (N=16)	5	2	2	1	0	1	5

The authors felt that the laser would be a very useful tool in future studies of insect behavior.

In medical research the laser microbeam has been employed by several groups in the study of microvascular circulation (14, 15, 26). Fine, Klein, and co-workers (14) ... conducted a series of laser microbeam experiments on microvessels as part of a study on the effect of laser radiation on the blood vessels, and possible relationships between those interactions and the gross effects of laser radiation on intact animals. . . . The system they used was a 100 mj. (nominal) TRG laser microbeam system, similar to that described above. Flaps of abdominal skin and exteriorized mesentery with retention of blood supply of anaesthetized mice (Swiss white, C57 black) were

irradiated at various energy levels. The smallest spot size obtained at the focal plane was approximately 5 microns in diameter. Some of the factors affecting the interaction were the energy level of the radiation, power density at the focal plane, spot size, and the diameter and shape of the blood vessel. At low energy levels, constriction of the blood vessel occurred, persisting for several minutes. At higher energy levels, blood flow within the vessel was arrested by the formation of an intravascular clot; although the integrity of the blood vessel wall did not appear to be altered. A further increase in energy caused aneurysmal dilatation at the site of the clot formation. Interstitial extravasation of blood was observed from the smaller vessels, and at sites of bifurcation. In preliminary studies on skin flaps, a Q-switched laser directed through the microscope produced hemorrhage, rather than thrombosis (27).

Kochen and Baez also used a TRG laser microbeam system to conduct a series of experiments on microcirculation (15,26,28,29). These studies were done with a 55X water immersion objective, which produced a spot 5 μ in diameter at the focal plane. The intensity of the laser beam was controlled with neutral density filters of known transmission, and was measured with a TRG ballistic thermopile mounted on the substage of the microscope. In order to obtain a dynamic record of the phenomena observed following laser irradiation, Kochen and Baez incorporated 16 mm. cinematographic facilities into the system (28). This series of experiments included studies on exposed rat meso-appendix (26), and studies employing 2 vascular model systems (28).

The findings of Kochen and Baez were in general agreement with those reported by Fine and Klein (14). Kochen and Baez (26) found that single laser pulses (0.5 up to 7.5 mj.) resulted in a progressive increase in vasomotion and a vascular hyperreactivity to epinephrine. The hyperreactivity to epinephrine stimulus increased with increasing laser energy output. A single microvessel showed a progressive increase in reactivity reaching a peak hyperreactivity in about 25 minutes after receiving a single 7.5 mj. laser pulse. These changes in vascular response were not accompanied by any discernible vascular or intravascular morphologic changes or thrombus formation. However, the injection of colloidal carbon particles into the blood stream resulted in the adherence of the carbon particles to the endothelial lining of the vessel wall in the region exposed to laser injury. Laser pulses of higher energy (7.5 - 15 mj.) resulted in transient "sticking" of platelets and leukocytes at the site of lasing, and the accumulation of carbon particles as much as 100 microns up stream and down stream from the lasing site. The deposition of carbon particles in this 7.5 - 15 mj range extended from the inner endothelial surface of the vessel through the perivascular space to the basement membrane and perivascular sheath. The extent and degree of carbon accumulation appeared to be independent of the rate of blood flow.

Laser pulses of 15-30 mj. resulted in maximally enhanced vasomotion and was consistently associated with the formation of a mural thrombus at the site of injury. Exposure of venules (30-60 u in diameter) to single laser pulses in the 15-30 mj. range resulted in the formation of a thrombus composed predominantly of platelets and leuko-

cytes, which often occluded the lumen of the venule. A progressive recanalization of the thrombus and subsequent dislodgement of fragments of cell mass and embolism followed within 2 minutes of maximum thrombus formation. Exposure of arterioles (30-45 μ diameter) to a laser pulse of 15-30 mj. intensity, resulted in a similar but more transient adherence and aggregation of platelets and leukocytes. Consequently, the thrombus had a tendency to be swept away as a microembolus. In neither arterioles nor venules did laser intensities under 15 mj. result in interstitial hemorrhage or distortion of the normal morphology of the outer vascular wall or surrounding tissue.

Focusing of a laser pulse of greater than 30 mj. intensity on the wall of an arteriole or venule resulted in the deposition of a fused red cell mass on the endothelial surface at the site of lasing, the evolution of a gas bubble which quickly disappeared, and the formation of an extensive and stable thrombus. Single laser pulses of 30-60 mj. intensity frequently resulted in localized regions of complete vessel wall breakdown and varying degrees of interstitial hemorrhage.

In an attempt to evaluate the effects of laser radiation on the vessel wall, per se, and on the elements of the blood, experiments were conducted on two model vascular systems (28):

(1) glass capillary tubing (60 u diameter) perfused with 0.9% saline solution colored sky blue and with heparinized rat blood.

(2) isolated, acutely denervated, rat mesoappendix vasculature perfused with mammalian Ringer's 3.7% albumin solution and heparinized rat blood.

Exposure of the glass capillary perfused with sky blue saline solution to a laser pulse of 30 mj. intensity did not produce any observable change in the flow characteristics of the saline solution. Glass capillary perfused with heparinized rat blood exposed to a laser pulse of similar intensity (30 mj.) resulted in the deposition of a fused red cell mass on the glass capillary and evolution of multiple gas bubbles. Exposure of isolated mesoappendix vessels to a 30 mj. laser pulse produced no apparent morphological change in vessels perfused with colorless Ringer's albumin solution; however, subsequent perfusion with heparinized rat blood resulted in the transient adherence of leukocytes and platelets to the site of previous irradiation. A single 30 mj. laser pulse produced a stable thrombus in isolated mesoappendix vessels perfused with rat blood at the time of irradiation. When the isolated vessels were perfused with colored Ringer's albumin solution, laser irradiation produced various degrees of damage, ranging from discreet cell adherence to complete breakdown of the vessel wall.

Kochen and Baez conclude from their studies that the vessel wall is involved in thrombogenesis, but the alteration initiating the adherence of blood elements to the vessel wall is not a change in wettability. These conclusions seem justified but not of immediate significance. The authors do point out the interesting fact that the changes in vascular reactivity they observed after laser irradiation of microvessels were similar to those observed following microelectrical stimulation of the hamster cheek pouch vasculature, which also was followed by thrombus formation (30). This observation is of interest, since, in

some cases the electric field may be of some importance. However, determination of the fields in tissue are not easily carried out, and may not correspond to those obtained by simple calculation for electric fields in vacuum. This is particularly true since the tissue is not at the focus, and rapid defocusing occurs. On the other hand, the opposite reaction, relaxation of vessel smooth muscle following exposure of vessels to light between 250 and 430 mμ in wavelength, has been reported (31).

It has been reported (32) that a group of doctors at Montefiore Hospital have been investigating the possible use of a laser beam to create anastomoses in small vessels. They use a $CuSO_4$ solution to stain the area of the vessels where the vessel union is desired; then they irradiate this spot with a neodymium laser. However, sufficient information for evaluating this technique is not available at this time, except as reported below.

A report by Strully et al on these studies has been presented (33). A donor and acceptor vessel were glued side to side. The common wall was then exposed to laser radiation by focusing through the open end of the donor vessel. There are obvious problems with this method. Since the donor vessel must be opened to permit penetration of the beam, a knife or fine cautery could have been just as readily used to incise the vessel wall. At the region of impact, temperature elevation occurs. This will result in thrombus formation and blood coagulation. The material which is removed by the irradiation will result in emboli. Blood coagulation at the site of irradiation will also result in emboli.

Although the technique has been presented in the medical press (32) as successful, no indication of this is evident.

While the microbeam is being used for the first time in entomology and microcirculatory research, microbeams have been used for partial cell irradiation (PCI) since 1912 (1). However, the introduction of the laser microbeam has stimulated an unprecedented interest in PCI.

Bessis and co-workers have made an extensive study of cell injury and cell death (9,11). Using lethal doses of laser radiation, then following the irradiated cell with time-lapse cinematography, a phenomenon referred to as "necrotaxis" (11) was observed. If a human red or white blood cell is irradiated, as soon as the irradiated cell shows any sign of alteration, the surrounding leukocytes travel toward it, attacking and phagocytizing the affected cell. However, on occasion the phagocytizing leukocytes will consume only part of the dead cell, abandoning the remaining portion of cell for some unknown reason (10, 11). This strange phenomenon bears further investigation, particularly on a biochemical basis, as it may yield pertinent information on the general phenomena of chemotaxis. Bessis has indicated that he has been exploring the possibilities of selective organelle destruction via differential staining to control absorption of laser radiation (10). However, no data have been presented to indicate successful differential staining for destruction of specific cellular organelles.

Saks and co-workers at N.Y.U. have explored the areas of microsurgery and partial cell irradiation with a TRG "Millilaser" microbeam system. Early in 1963, Saks and Roth (13) reported their initial

studies with a laser microbeam. They successfully made openings of approximately 25 microns in the cell walls of the alga, Spirogyra, without causing other gross irreversible damage. This facilitated microsurgery to be performed on some internal structures of the cell without doing irrepairable damage to the exterior cell wall. The laser used for these early studies had a maximum energy output of 20 mj per pulse and a pulse width of 5×10^{-4} seconds; however, even with these energy levels it was possible, because of the energy and power densities, to sever the chloroplast helix, disturb intracellular crystals, and to produce localized coagulation of the chloroplasts and cytoplasm. Leppard, has also been able to remove chloroplasts from a variety of plant cells, using a similar system (5). Saks and Roth reported (13) that irradiation of the cell nucleus with an incident beam energy of 0.08 mj per pulse produced no discernible effects. The focusing of higher energy laser pulses on the nucleus resulted in coagulation and a reduction of nuclear volume. A grossly similar nuclear reaction is caused by ultraviolet light (34). Saks and Roth (13) also found that addition of a low concentration (0.01%) of Methylene blue chloride, which localizes on the cell wall, appreciably reduces the energy required to produce alterations 15-25 μ in diameter on the cell wall. Similarly, irradiation of the chloroplast helix with 0.3-0.6 mj resulted in coagulation of chloroplasts and cytoplasm, loss of turgor pressure, and a rounding of the nucleus.

Additional studies conducted by Saks et al (16, 35) have resulted in some improvements in technique, and have produced some in-

teresting results. Amoeba proteus and Nitella hyalina were used for these studies. The laser microbeam used was an improved version of the J.G "millilaser" microbeam system with a maximum laser output of 100 mj per pulse. The laser was focused by a 16X ocular and a 10X, 32X or 43X objective (16), or a 20X ocular with a 58X objective (35). The later system had a minification factor of 1450X to give a spot 4.3 microns in diameter. Energy levels were controlled with metalized, Bauch & Lomb, neutral density filters of known transmittance.

Saks has worked out a simple procedure to align the laser with the optical axis of the microscope (16). A thin film of green dye is allowed to dry on a microscopic slide. As the slide dries, small cracks are formed in the surface of the dye; these cracks can be used to focus the microscope to insure the slide is in the focal plane. The laser is fired producing a hole in the dye. The alignment necessary to obtain a symmetrical spot in the proper position can be done. The alignment is checked again using the same procedure, until the laser is exactly aligned. The advantage of this system over others using metalized slides or carbon paper is the cracks formed in the dye; these provide an outstanding landmark on which to focus the objective. This is important if one wishes to obtain the smallest spot possible for a given ocular and objective.

Studies on Nitella (16) demonstrated that the laser microbeam was an effective microsurgical tool which complemented the micromanipulator and microinjection systems. Irradiation of the internodal cell wall near the node, three times, with a 156 μ microbeam

with incident energy of 12.5 mj per pulse considerably altered the cell wall at the site of irradiation. This area provided a "window" in the cell wall through which micropipettes could be inserted to inject various materials or perform other microsurgical manipulations. Previously, the cell wall had greatly hindered microsurgical experiments on plant cells. Irradiation with 58X objective with a 20X ocular (35) produced holes in the cell wall of Mitella thus permitting the insertion of a micropipette. (The mechanical withdrawal of the micropipette produced more damage than the laser beam.) The laser beam minified by a factor of 1450X in this system fused, 8-16, chloroplasts and caused only restricted local damage.

The effect of laser irradiation on Mitella growth rates after receiving 1, 3, 5, or 7 pulses of 12.5 mj from the incident beam, with a beam diameter of 156 u (19.5 μ in experiment 2) was studied. A single pulse produced browning and blackening of cell wall in the target area similar to that seen in Spirogyra (13), and chloroplasts were dispersed or coagulated in an area about 64 u in diameter around the target site. Cyclosis was stopped for a period of time that increased with the number of times the Mitella was irradiated. In those Mitella receiving only a single pulse, the cyclosis resumed within 2 to 5 minutes after irradiation, and in those receiving 7 successive pulses of laser radiation, cyclosis resumed within 12 to 15 minutes following irradiation. The growth rate of all the irradiated plants was less than that of the controls; however, the per cent decrease did not correspond to the number of irradiations.

Laser microbeam studies on Amoeba proteus (16, 35) are of particular interest since a number of ultraviolet and high energy microbeam

studies of Amoeba have been reported in the literature (2, 3). Saks and his associates irradiated Amoeba with laser pulses of 20 mj and 100 mj per pulse at pulse repetition rates of 1 pulse per minute and 1 pulse per 3 minutes, with beam diameters of 500 μ, 116 u and about 5 μ (16, 35). They found Amoeba to be very resistant to laser radiation (16); this also occurred with other types of radiation (3). Saks reports that following repetitive irradiation of the cytoplasm at 1 pulse per minute at 20 mj per pulse, the Amoebae go through a cycle following each pulse:

1. Brief latent or undisturbed period.
2. Enhanced forward cytoplasmic streaming.
3. Enhanced reverse cytoplasmic streaming.
4. Local shift in sol-gel equilibrium toward solution, referred to as "ballooning".
5. Recovery.

Average recovery time was 8-9 seconds (16). This description of the Amoeba's response to laser microbeam irradiation is similar to that reported by Tchakhotine in 1935, after irradiation of Amoeba with his 2800 Å microbeam (36). Accumulative changes in the sol-gel state of amoebae resulted from repetitive 20 mj pulses of laser radiation (16). This "ballooning" was detectable by the progressive loss of the ability to form and maintain pseudopodia; in all cases the Amoebae were able to recover from this "ballooning" phenomenon. Saks suggests that this accumulative damage may indicate that the lipoproteins and proteins of the sol-gel system are practically denatured and accumulate with repetitive irradiation.

Irradiation of amoeba cytoplasm with a 100 mj incident laser beam immediately produced rapid solution, violent contraction of the whole animal, and recovery. If the cytoplasm was irradiated repeatedly with 100 mj pulses there was accumulative damage. Frequently, the cytoplasm in the target area was pinched off, and occasionally fragmentation of the amoeba followed. In addition, irradiation of amoeba cytoplasm with 100 mj pulses caused the specific ejection of food vacuoles, especially if the ingested food contained chlorophyll (16). R. Zuzolo and Saks have reported (35) that if amoebae are injected with microdrops of mineral oil, olive oil, or olive oil containing Nile Blue Sulfate and the oil drops irradiated with the 116 μ micro-laser-beam, the oil drops are frequently ejected. However, irradiation of intracytoplasmic oil drops using the 20X ocular and 58X objective to obtain a minifaction factor of 1450X (i.e., beam diameter approximately 5 μ) did not elicit such drastic effects. There was only an accumulation of cytoplasmic debris at the oil-cytoplasm interface, without ejection of the oil drop. If the amoebae were vitally stained with Methylene Blue, or Nile blue Sulfate was microinjected into the cytoplasm after the oil drop was injected, radiation from the 5 μ laser microbeam usually produced pinching off or ejection of the stained cytoplasm and the micro-oil drop (35). The "pinching off" of irradiated cytoplasm has also been observed following the irradiation of amoebae with a uv microbeam (37).

Saks and his co-workers (16, 35) also studied the effect of the laser microbeam on amoebae if only the nucleus was irradiated. Repeated irradiation of nuclei with 20 mj incident beam with a 116 μ spot size produced behavior distinctly different from those amoebae whose cytoplasm was irradiated. Irradiation of the nucleus resulted

in a sequence of events beginning with the immediate inhibition of cytoplasmic streaming. The inhibition of cytoplasmic streaming was followed by a reverse granular flow (cytoplasmic streaming opposite to that in non-irradiated or recovered amoebae). This was followed shortly by an enhanced cytoplasmic streaming in the original direction or in a different direction. In addition, solation and spasmodic cytoplasmic streaming was evident, and the rate at which the amoebae advanced pseudopodia were reduced. When irradiation was discontinued, the amoebae were able to recover and reproduce, if damage to the nucleus was minimal.

Irradiation of nuclei with 100 mj from the incident beam resulted in a sudden stoppage of cytoplasmic streaming followed by violent rapid solation and contraction of the whole amoeba, after which the amoeba recovered. Repetitive irradiation of the nucleus with the 100 mj incident beam caused irreversible damage. This resulted in a microscopically effectively enucleated amoeba, although the nucleus was not ejected by the amoeba. The amoebae became more spherical, cytoplasmic streaming became limited, and pseudopodia were not formed. The effects are compared to those following enucleation with a microneedle. If, however, the amoebae were vitally stained with Methylene Blue, nuclear irradiation with the 100 mj laser microbeam caused the nucleus to be ejected.

Following successive irradiations (1 to 7) of nuclei and cytoplasm of amoebae at incident energy levels of about 100 mj and a beam 116 μ in diameter, irradiated amoebae were cloned with non-irradiated

controls, in order to determine the effect of a laser microbeam on the miotic and growth rates of amoebae. In all cases the irradiated amoebae showed a decrease in miotic rate as compared to the control colonies. The maximum decrease appeared after 5 pulses of laser radiation. Five pulses also resulted in maximum decrease in the growth rate of Nitella (16).

Initial microbeam studies on the formed elements of the blood were carried out by Fine, Klein, and co-workers (14). The general morphology and amoeboid movement of isolated, viable human granulocytes were not affected by the 6943 Å laser radiation, using a TRG microscope laser system. However, the addition of Methylene blue (final concentration 10^{-4}%) markedly altered the effects of irradiation. Depending on the site and energy of irradiation leukocytes in Methylene blue solution were partially or entirely fragmented, and even when a semblance of structural integrity was retained, amoeboid movement was lost. Similarly, platelets showed no apparent effects from laser radiation; however, in the presence of Methylene blue, laser irradiation resulted in fragmentation of the platelets accompanied by the release or formation of granular material. In contrast, isolated viable human erythrocytes contracted into irregularly shaped bodies 2-5 μ in width in various planes following irradiation with the laser microbeam. These observations are in agreement with others who have reported laser microbeam studies on the formed elements of the blood (8, 6).

Other data reported by Fine, Klein et al (14) was the effect of the laser microbeam irradiation on alligator erythrocytes. Alligator erythrocytes are elliptical, approximately 10 μ x 30 μ in size, and

contain a nucleus. They found that irradiation of only the cytoplasm could affect only the cytoplasm at the target site, while the nonirradiated parts of the cell remained relatively unchanged. In contrast, irradiation of the nucleus with the laser microbeam caused the alligator erythrocytes to contract into a multilobed structure about 1/3 the pre-irradiation size. This observation is of particular interest in view of Saks' report (16) that the response of amoebae is much more violent when the nucleus is irradiated, as opposed to irradiation of the cytoplasm only.

It is doubtful that these studies indicate that nuclear integrity is significant for maintaining cellular integrity. The difference in the effects produced is probably due to differences in the absorption characteristics of the tissues, in particular, differences between the cytoplasmic and nuclear constituents. Although various constituents may have similar absorption characteristics, the relative concentration of these would affect the total energy absorbed per unit volume. It should be noted that although beam energy measurements were made in some instances, actual absorption measurements were not carried out. Absorption measurements would be particularly difficult to carry out at high peak power densities on single cells, since such factors as reflection and scatter must be considered. Furthermore, the beam geometry is not constant, but varied markedly, both with distance from the focal point, and with scatter and absorption. The spot sizes discussed must be considered as approximate, since it is based on effects on a specific material that is used as a standard. It probably represents an area of relatively high power density.

It is probable that a more sophisticated system would provide a more satisfactory microbeam geometry for some purposes. The system would consist of a set of lenses which would converge the beam into a relatively parallel beam much smaller in diameter than the original beam, and would be independent of the optical system of the microscope. A system of this type could incorporate either gas, semiconductor or solid state lasers. It would have the disadvantage that the energy density would be constant, whereas with a focused system, the energy density can be low relative to the energy density in the region of interest. However, since the focal point of the microscope laser system and the location of the target do not coincide exactly, the region of maximum intensity of the beam is not at the point in focus through the microscope, and is difficult to compensate for, rapidly and easily. A relatively parallel beam of very small cross section would also be difficult to maintain in air, over any distance and time, because of the high field strengths and consequent irritation produced.

Lasers have been successfully combined with microscopes for use in other areas of biological research including the study of cell respiration (38), embryology (18,39) and emission spectroscopy (40). These applications are covered in greater detail in other sections of this report. Laser-microscope units have also been developed for such industrial applications as micro-welding and micro-drilling (41, 42). These units are operational now . However, such applications, though interesting, are not of sufficient biological importance to be considered in detail here. Hazards associated with these units will require further study.

It appears that the laser-microscope is an experimental tool which could facilitate a variety of worthwhile studies in many different areas of bio-medical research. However, there are many problems which need to be solved before the laser microbeam will yield quantitative and useful information, not obtainable by other means.

Perhaps the most important problem to be solved is the development of adequate monitoring systems. The investigator needs a record of several parameters, including the energy density at the focal point, the total energy incident on the sample, and the total energy absorbed by the sample. Incorporation of a beam splitter in the microscope tube, to sample a small percentage of the laser beam during irradiation is feasible. Sampling of the beam between the objective and target is difficult unless long focal length objectives are used. The energy transmitted could be estimated by placing a photoelectric detector on the substage. It is necessary that a relatively standard measurement system be used in microbeam studies so that data can be compared with that of other investigators working with laser microbeams, and with the observations made with non-laser microbeam systems.

Incorporation of closed circuit TV into the system, as used by Bassis (10), would enable investigators to observe phenomena during and immediately following irradiation, to some extent, without exposing themselves to the associated viewing hazards. Since the pulse duration is short, and resolution limited, the advantages may not be as pronounced as expected. Video tape recording can, of course, be included.

Regardless of the individual weaknesses in any of the laser microbeam studies that have been reported, they have all indicated areas which should yield a great deal of worthwhile information. The studies

of Mitt and his associates (24) on spider behavior have shown that the laser microbeam can be a valuable tool, not only in entomology, but more importantly in the general area of animal behavior. Bessis studies (10, 11) on cell injury and cell death could provide information on the mechanism of chemotaxis.

Sabs' work with Spirogyra and Mitella (13, 16) have ably demonstrated the usefulness of the laser microbeam in microsurgery. The studies on alligator erythrocytes by Fine and Klein (14) and Sabs studies on amoebae (16) have given at least some indication that partial cell laser irradiation, to study the importance of various subcellular organelles, is feasible. However, no one has yet demonstrated a means of carrying out selective destruction of specific organelles. The use of specific dyes has been suggested (17), but data has not been reported. Bessis stated that he has successfully destroyed, in a selective manner; mitochondria after staining with Janus green, and golgi bodies dyed with Nile blue; however, no data are yet available for evaluation. Other possible means of selective destruction of cell organelles include the use of lasers with outputs at specific frequencies corresponding to some absorption band of a compound contained only in one particular type of organelle. Another possibility which should be considered is the use of polarized laser microbeams and the principles of photoselection (43) to take advantage of the birefringence observed in certain protein (44) and in some nuclear DNA (45). In relation to the concept of selective destruction it would be of interest to carry out further studies to confirm Sabs' observation that very small beams, 5 μ or less, produce only confined, localized damage in the area of the

target site.

The technique could be of value in determining the mechanisms involved in the interaction of laser radiation with biological systems. Since there is a good background of microbeam research using other sources of radiation (2, 3) it may be possible to compare the effects of other types of radiation with the effects caused by laser microbeams on selected organisms or components. It would also be relatively easy to do comparative studies at a cellular level. The value of microbeam studies in elucidating effects on the intact animal is partially indicated by the microvascular studies of Kochan and Baez (26, 28) and Fine, Klein, et al (14), which demonstrated the ability of lasers to produce vascular changes, including hemorrhage and thrombosis.

The cost of laser microbeam units for research would vary between $3,000 and $25,000 depending on the complexity of the microscope laser system, and the associated equipment including micromanipulators, TV viewing facilities, and recording cameras.

REFERENCES

1. Tschachotin, S., Biol. Zentr. 32:623 (1912).

2. Zirkle, R.E., Partial-Cell Irradiation, Advan. Biol. Med. Phys. 5: 104-146 (1957).

3. Smith, C.L., Microbeam and Partial Cell Irradiation, Internat. Rev. Cytol. 16:133-153 (1964).

4. Townes, C.H., Optical Masers and their Possible Applications to Biology. Biophys. J. 2 (pt. 2):325-329 (1962).

5. Malt, R.: Effects of Laser Radiation on Subcellular Components, Fed. Proc. 24(1, pt. 3) suppl. 14:s122-125 (1965).

6. Peppers, N.A., A Laser Microscope, Appl. Optics, 4:555-558 (1965).

7. Bessis, M. and G. Nomarsky, Irradiation Untra-Violet des Organites, J. Biophys. Biochem. Cytol. 8:777-791, (1960).

8. Bessis, M., Gires, F., Mayer, G., Nomarsky, G., Irradiation des Organites Cellulaires a l'aide d' un Laser a' Rubis, Compt. Rend. 255:1010 (1962).

9. Bessis, M. and Burte, B., Chimiotactisme Apres Destruction d'ure Cellule par Microfaisceaux Laser, C.R. Soc. Biol. 158:1995 (1964).

10. Bessis, M. and Ter-Pogossian, Micro-puncture of Cells by Means of a Laser Beam, Ann. N.Y. Acad. Sci. 122:689-694 (1965).

11. Bessis, M., Studies on Cell Agony and Death:an Attempt at Classification, Ciba Foundation Symposium, Cellular Injury, edited by A.V.S. de Reuck and J. Knight, (Boston: Little, Brown & Co., 1964), pp. 287-328.

12. Fine, S., Klein, E., Hardway, G., Scott, R.E., King, W. and Aaronson, C., The Use of Closed Circuit Television in Laser Investigations, J. Invest. Dermatol. 42:289-291 (1964).

13. Saks, Norman, M., Roth, Charles, A.: Ruby Laser as a Microsurgical Instrument, Science 141:46-47 (1963).

14. Fine, S., Klein, E., Nowak, W., Scott, R.E., Laor, Y., Simpson, L., Criscey, J., Donoghue, J. and Derr, V.E., Fed. Proc. 24(1,pt. 3) suppl. 14:s35-45 (1965).

15. Baez, S. and Hochem, J.A., Effects of a Pulsed Laser Micro-Beam on the Microcirculation, Fed. Proc. 23:253 (1964).

16. Sako, H.M., Zuzolo, R.C. and Kopac, M.J., Microsurgery of Living Cells by Ruby Laser Irradiation, N.Y. Acad. Sci. 122:695-712 (1965).

17. Walt, R.A. and Tomes, C.E., Optical Masers in Biology and Medicine, N. Engl. J. Med. 269:1417-1421 (1963).

18. Lang, K., Barns, F.S., Daniel, J.C. and Meisel, J.C., Lasers as Tools for Embryology and Cytology, Nature 201:675-677 (1964).

19. Tomberg, V., Non-Thermal Biological Effects of Laser Beams, Nature 204: 868-870 (1964).

20. Tomberg, V., Biological Effects of Concentrated Laser Beams, in Optical Masers (Brooklyn, N.Y.: Polytechnic Press, 1963) pp. 505-508.

21. Lacquin, P.M., Lasers et Microscopes, Z. Wiss. Mikr. 66:68-72, (1964).

22. Article in Laser Focus 1(3):6-9, (1 February 1965).

23. Data Corporation, Dayton, Ohio; report available from Spacerays, Inc. 220 East 42nd Street, New York, N.Y. 10017

24. Witt, P.N., Reed, C.F. and Tittel, F.K., Laser Lesions and Spider Web Construction, Nature 201:150-152 (1964).

25. Fine, S. and Klein, E., Biological Effects of Laser Radiation, Amer. Biol. Med. Phys. (1965) in press.

26. Kochen, J.A. and Baez, S., Laser Induced Microvascular Thrombosis Embolization and Recanalization in the Rat, Ann. N.Y. Acad. Sci. 122: 728-737 (1965).

27. Klein, E. and Fine, S., Effects of Laser Radiation on Animal Tissues, presented at Conf. Laser, N.Y. Acad. Sci., New York (1964).

28. Baez, S. and Kochen, J.P., Laser Induced Microagglutination in an Isolated Vascular Model System, Ann. N.Y. Acad. Sci. 122:738-746 (1965)

29. Kochen, J.A. and Baez, S., Vascular and Intravascular Effects of a Pulsed Laser Micro-beam, Proc. III Europ. Conf. Microcirc., Israel J. Exptl. Med. 11:109 (1964).

30. Callahan, A.B., Lutz, B.R., Fulton, G.P. and Degelman, J., Smooth Muscle and Thrombus Thresholds to Unipolar Stimulation of Small Blood Vessels, Angiology 11:35 (1960).

31. Burchgott, R.F., Ehrreich, S.J. and Greenblatt, E., The Photoactivated Relaxation of Smooth Muscle of Rabbit Aorta, J. Gen. Physiol. 44:499 (1961).

32. Yahr, W.Z., Strully, K.J. and Hurwitt, E.S., Paper presented at meeting American College of Surgeons, Chicago, Ill. (1964); as reported in Medical World News, p. 68 2, (Nov. 6, 1964).

33. Strully, K.J., Yahr, W.Z. and Hurwitt, E.S., Experimental Cardiovascular Laser Surgery: a Method for Small Blood Vessel Anastomosis Utilizing Laser and Monomer, Proc. Biomed. Laser Conf., Laser Med. Res. Found., (June 1965).

34. Montgomery, P. O'B., Ann. N.Y. Acad. Sci. 97:491 (1962).

35. Zuncle, R., Saks, M.M. and Hopic, M.J., Studies with a Microscope Laser Unit, Proc. Boston Laser Conf., 3rd, Boston, Mass. (Aug. 1964).

36. Tchakhotine, S., Compt. Rend. 200:2217 (1935).

37. Grodzinsky, Z., Acta. Biol. Gracov. Ser. Zool. 4:47-57 (1961).

38. Hamberger, A. and Tengroth, B., The Q Switched Laser as a Tool in the Micro-Diver Technique, Expt. Cell Res. 37:460-463 (1965).

39. Barnes, F.S., Some Applications of Lasers to Embryology and Biological Data Processing, Proc. Biomedical Laser Conf., Laser Med. Res. Found., (June 1965).

40. Rosan, R.C., Healy, M.K. and McNary, W.F., Jr., Spectroscopic Untramicroanalysis with a Laser, Science 142:236-237 (1963).

41. Sherman, R.A., Microwelding and Microdrilling with Lasers, Ann. N.Y. Acad. Sci. 122:650-657 (1965).

42. Rischill, H., Laser Welding of Micro-electric Interconnections, IEEE Trans. Component Parts CP-11(2):145-152, (June 1964).

43. Albrecht, A.C., Polarized Light in Vibronic Spectroscopy and Photo-Chemistry, Proc. Symp. Molecular Structure and Spectroscopy, Ohio State University, Columbus, Ohio, (June 15, 1965).

44. Taylor, T.W. and Cramer, W., Birefringence of Protein Solutions and Biological Systems, I and II., Biophys. J. 3:127-154, (1963).

45. Inoué, S. and Hideni, S., Arrangement of DNA in Living Sperm: A Biophysical Analysis, Science 136:1122-1124 (1962).

Embryology

The blastoderm of unincubated fertile chicken eggs were irradiated with a ruby laser rated at 0.1 joule output laser energy by Lang et al (1). The beam was focussed to blastoderm size by a lens, the focal length of which terminated at embryonic depths within the eggs. Irradiation of the blastoderm in these cases required penetration of the shell, shell membrane and several millimeters of albumin. Following incubation for 22 days, deformities including splay legs, a club foot and visceral protuberance were obtained. Further experiments were carried out by windowing the eggs with a sterile cover slip to facilitate direct focussing of the laser on the blastoderm. All controls that survived to 72 hours were normal. None of those receiving 2 shots of the "low energy" beam appeared normal. Only the heart and associated vessels were identifiable as a tissue organ system following irradiation, the other embryonic structures could be identified only in traces.

These results indicated that teratogenic effects could be produced at 6943 $\mathring{A}$, at laser power and energy levels. The input energy to the laser at which the irradiations were carried out are listed. However, since lasers have a threshold, and are essentially non-linear insofar as output versus input is concerned, the energy of irradiation requires documentation, for more meaningful future studies. There is no indication that the refractive index of the media was taken into account on focusing. The measurement of scattering in this heterogeneous system is difficult. These factors would affect the energy and energy density incident on the blastoderm. Since the number of eggs used was relatively

small, the statement that the number of deformities (expressed as percentage of eggs irradiated) is roughly proportional to the energy received by the eggs requires further data and study. It is interesting that at high energies the number of eggs hatching (45.4%) was almost equivalent to that of the control 50%. At much lower energy output levels the number hatching was significantly less. Its significance relative to the deformities produced required further consideration. This was essentially an excellent preliminary report and it is presumed that more extensive studies will be carried out.

In the 19th century two concepts formed an important background for work in the field of experimental embryology. These concepts were the concept of mosaic development as advanced by Roux in 1888 and the concept of totipotency as advanced by Dueseh in 1894. The first of these led to the studies on localization and mapping of prospective areas by Vogt 1925 (et al) while the second led to the production of postulates defining totipotency. Experimental evidence led to the realization that a series ranging from absolute mosaic development to absolute totipotency existed rather than either extreme.

Morgan in 1895 found that inversion of the amphibian egg or removal of the damaged blastomere would preclude mosaic development. Studies were conducted by Spemann in 1938 on the potentialities and interrelationships of the two halfs of an egg. The emphasis of the studies thus shifted from the potency to the mechanics of organization during development. Nicholas and Hall and Tarkawski, carried out studies on isolation of mammalian blastomeres.

Laser studies were carried out by Daniel and Tajahashi (2) Fertilized ova from rabbits were flushed from the ova ducts with F-10 culture medium and collected in sterile watchglasses. A 2-cell state was obtained at 22 to 26 hours postcoitum a 4-cell stage at 30 to 34 hours postcoitum, and 8-cell state at 38 to 42 hours postcoitum and a 16-cell stage at 46 to 52 hours postcoitum. (Cleavage was irregular after the 4-cell stage).

The ova were stained with methylene blue in a concentration of 0.05 mg. per cc. which was previously determined to be nontoxic to the ova insofar as cleavage is concerned. Following staining, the ova were washed once in stain-free medium, supported in a hanging drop of medium, positioned under a microscope, and a single blastomere brought into focus. Following lasing of the blastomere, (at 3 millijoules output from the microscope) it collapsed within minutes into a amorphous mass. Generally, each blastomere was lased separately. As the zona was not broken, both the destroyed and the surviving normal blastomeres were retained within it. Following irradiation, the ova were cultured in vitro.

Cleavage of the surviving blastomeres were noted, and followed until controls of unlased ova reaches the 32-to64-cell stage. At 8-and-16-cell stages, it was difficult to obtain sufficient isolation between the cells on lasing. In three cases they did manage to destroy all but one cell in the 16-cell stage. The output energy was measured with a ballistic thermocouple.

Following irradiation at exit energies from the microscope of 3 millijoules, the unlased cell assumed a more spherical shape, considered as possibly due to decreased pressure on it by its coblastomeres. (However, the alteration in the chemical, as well as physical environment must be considered in explaining this effect).

Cleavage of the surviving blastomeres occurred. The surviving blastomere in the 2-cell stage cleaved regularly to produce 16 cells

in the 4-cell stage to produce 8 cells, and the 8-cell stage to produce 16 cells, but not regularly. In the 16-cell stage, the surviving blastomere cleaved once in one case, and in the other two cases, twice. With decreased staining or irradiation energy, a general slowing of the rate of cleavage occurred. Inhibition of cleavage by visible light has previously been reported by Daniel (3).

Attempts were made to calculate the temperature pattern within the cell, assuming a homogeneous energy absorption. The effects were considered as primarily thermal, rather than due to electric fields.

The result indicated that a surviving rabbit blastomere may grow and divide as well in vitro as intact ova. The authors indicate that it does not show independent totopotency of one of the first eight or of the first sixteen blastomeres of the rabbit. Reference is made to Seidel who has obtained a narmal viable young rabbit following transplantation of an ovum after destruction of one of the first two blastomeres with a hot needle (4).

The possible use of a laser as a tool in embryology has been described. It may possess advantages over a hot needle which has been used for destroying blastomeres. Although alpha particle irradiation can be attempted, its penetrability is poor. Ultrasonic radiation probably cannot be easily focussed to irradiate a small region without damage to adjacent areas. Since low energy is required, the use of gas lasers may provide a more satisfactory method, since they can be focussed to a smaller

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BIOLOGICAL EFFECTS OF LASER RADIATION. VOLUME I. REVIEW OF THE --ETC(U)
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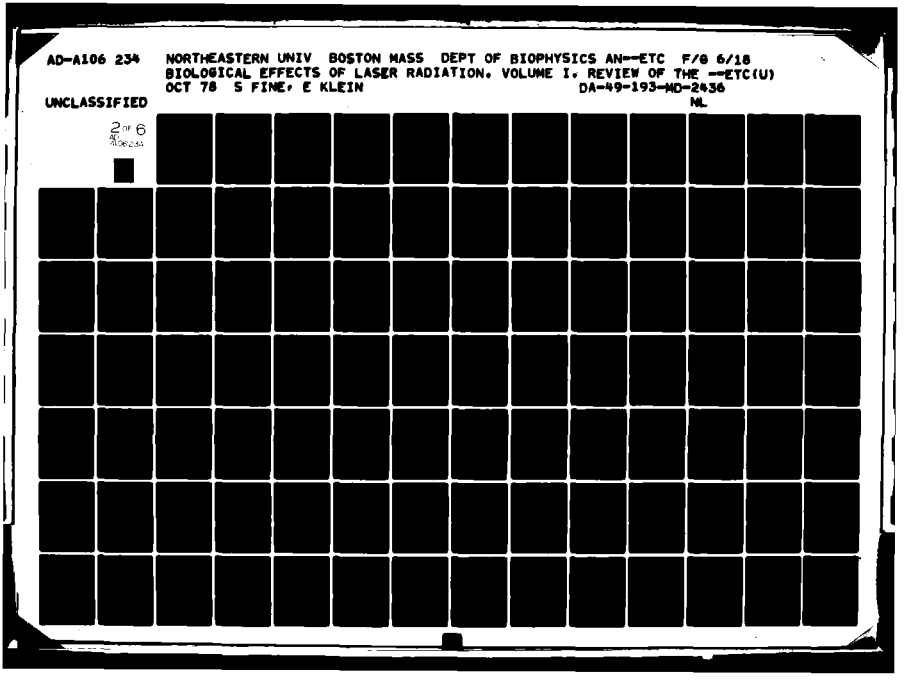

spot size, and permit the use of other wavelengths. This may permit selective destruction without staining, or with less staining than used in the above studies.

Studies by Edlow et al (5,6) were undertaken to determine whether laser induced alterations could be produced in mammalian embryos and fetuses directly in utero without rupture of the uterus or leakage of the amniotic fluid, and if this should be possible, to characterize the nature of fetal tissue response to localized injury.

Localized lesions were produced in the mammalian rat embryo and the fetus following focussed laser irradiation (6943Å, millisecond, pulse duration) through the intact uterus which was delivered through a ventral abdominal incision. An intrauterine fetal lesion was produced, although not in all cases, without rupture of the uterus and amniotic sac, and without gross leakage of amniotic fluid. In some cases, the uterus was opened following irradiation and the fetal lesion examined grossly and microscopically. In other instances, following irradiation late in gestation, it was possible to produce a localized lesion in a fetus in utero which was similar to a lesion observed in one of the litter-mates when the pregnant animal delivered normally per vaginum 36 hours later. In some of the embryos, resorption was observed several days following irradiation.

Generally, fetal lesions were significantly larger and more intense than those produced earlier in gestation. Microscopic examinations of the fetal lesions showed dilation of vascular

channels with marked congestion and extensive disruption and fragmentation of fetal tissue. These changes were especially prominent at the boundaries or surfaces between adjacent and frequently dissimilar tissues. These included the interface between cartilage and muscle, muscle and skin, muscle and subcutaneous tissue, and skin and subcutaneous tissue. The latter tissues were most frequently involved. Maximum disruptive lesions appeared in the plane of the laser beam. Fetal skin showed no evidence of carbonization. The skin of one fetus, however, showed epidermal coagulation. The skin of several others showed intra-epidermal edema with marked pyknosis of the nuclei of reserve cell layers. All of these lesions were in the tract of the laser beam. On several occasions, marked histopathologic alterations occurred in internal viscera in the path of the laser beam. This involved necrosis of hepatic parenchymal cells in two fetuses and intense but focal pulmonary congestion in another.

The importance of factors such as tissue and organ interfaces of differing physical and chemical properties and of possible pressure waves associated with changes in phase within the tissue were considered.

Localization of the site of irradiation presented a problem. The possibilities of obtaining better localization especially in embryos, with the use of normal pulse duration or Q switched lasers coupled through microscopes, with suitable focal length objectives was indicated.

These studies by Edlow et al. indicated that production of localized lesions in a mammalian fetus through the intact uterus, by laser radiation could be acheived. The comparative response to local injury by fetal, neonatal and adult animals is of basic interest, and is being currently pursued.

It is not possible to well localize and delineate the interaction, because of problems of visualization and the inherent properties of the radiation and target. Measurement of actual energy and energy densities within the tissues will continue to present problems. The effect of anaesthesia must be considered. Localized irradiation and surgical procedures on the rat embryo in early pregnancy is probably not feasible. Other mammalian species may provide a better basis for production of localized superficial lesions in the embryo and fetus with minimal injury to the uterus, ammion and other tissue.

The method may prove superior to that of the hot needle, if techniques for localization can be achieve. It will have to be compared with results obtained with ultrasonic irradiation of fetal tissue.

1. Lang, K. R., Barnes, F. S., Daniel, J. C., Maisel, J. C., "Lasers as Tools for Embryology and Cytology", Nature, 201, (4920):675, 1964.

2. Daniel, J. C. and Tajahashi, K. "Selective Laser Destruction of Rabbit Blastomeres and Continued Cleavage of Survivors in Vivo" (to be published in Experimental Cell Research).

3. Daniel, J. C. Nature (London). 201, 316 (1964).

4. Seidel, F. Naturwissenshaft. 39, 355 (1952).

5. Edlow, J., Fine, S., Vawter, G. F., Jockin H., Klein, E. "Laser Irradiation: Effect on Rat Embryo and Fetus in Utero" Life Sciences 4 (5):615:623, 1965.

6. Edlow, J., Farber, S., Fine S., Klein, E. "Prenatal and Neonatal Effects of Laser Radiation", Abstracts of Biological Sessions, Boston Laser Conf., August, 1964.

STUDIES ON NORMAL ANIMALS

Studies on intact animals are required for an understanding of the in vivo interaction of any agent, whether it be physical, chemical or biological in nature. Animal studies provide data which, in addition to their inherent significance, form the basis for studies in humans. Further information concerning the phenomena observed is obtained by studies on biological systems in vitro, such as isolated molecules of biochemical significance, separated tissue components, or tissue preparations, and model systems. Studies on experimentally induced pathological states, such as transplanted tumors in animals become meaningful when compared to studies on the normal state, as determined by the vivo and in vitro investigation. Some of the information gained may then provide a background for application to clinical problems in man. When new modalities become available for bio-medical research, problems arise due to the lack of background information such as is available in established fields of research. This lack of information may be more readily replaced by unfounded hypotheses than can be introduced into areas which have been circumscribed by serious scientific efforts. At the same time, it is more difficult to evaluate investigational approaches in a new field because specific and definitive background data is lacking.

The clinical scientist is usually unfamiliar with the general aspects of the interactions of physical agents with biological systems. The physical scientist, in turn, who has

had limited contact with biological studies is not cognizant of the problems involved in biological and clinical research.

Some of the literature directed towards the clinical aspects of laser radiation discuss preliminary results, in which minimal consideration is given to preceding or concurrent animal studies. Verbal presentations, either given at meetings or through the medium of news releases without the benefit of scientific editorial review have resulted in the dissemination of some but not all factors associated with the observations, and thus have provided a basis for misinterpretation. Even when presented through acceptable channels of scientific communications, a more orderly sequence of studies would be desirable. Thus a number of reports on studies of laser effects on experimental tumors would have benefited from preceding or accompanying studies on normal animals. Since many experiments were performed with prototype, unreliable equipment, some studies were not well controlled, and cannot be statistically evaluated. Interpretations, extrapolated from few observations may be lacking in scientific information. The overall effect, therefore, is not only a failure to provide scientific information, but can result in misorientation of future work, including extensions to clinical applications which may not be as meaningfully pursued as would otherwise be possible and which may lead to unjustifiable hazards to patients.

For these reasons, which are being documented throughout this report, studies on normal animals require particular attention. The general purpose of these studies may be summarized as follows:

1. To investigate the short term and long term effects of the interaction of laser radiation with skin and underlying normal and tumor (discussed separately) tissue.
2. To determine thresholds for gross and histological changes.
3. To evaluate the hazards associated with laser radiation.
4. To orient subsequent studies of the biological interaction of laser radiation as available at present and in the future with biological systems, both in vivo and in vitro.
5. To provide a basis for clinical applications of laser radiation.

Comprehensive studies were carried out by Fine, Klein and coworkers (1 - 23). Studies included an investigation on 6,000 mice in addition to required control animals. Exploratory studies by these authors included several mammalian species, such as rat, hamsters, rabbits and monkeys. These studies were integrated with in vitro studies on biological preparations (7,9-11,13,14,16,17-19,21,24), and with studies on the physical

and chemical aspects (7,9-14,16-19, 21-24) of the interactions of laser radiation with biological systems. Most of these studies were carried out with pulsed lasers at 6943 Å and 10600 Å. Exploratory (as yet unreported) studies on more than 100 animals were carried out with an ionized (continuous) argon laser at power levels of up to 5 watts. Preliminary studies were carried out with continuous Ga As semiconductor lasers in the 100 milliwatt range which were constructed and adapted at Northeastern University for biological work. Biological studies with radiation obtained from C.W. He-Ne (1 mw at 6328 Å) and low power pulsed Nitrogen gas lasers (3477 Å) did not show changes in the in vivo systems under investigation.

In the studies with pulsed solid state lasers, the parameters of the radiation were varied to the extent feasible at the time. Energy levels ranged from the millijoule level to more than 1000 joules per pulse. Corresponding power levels ranged from the watt region to more than 500 MW non-Q-switched and more than 500 MW Q-switched. Corresponding energy and power densities depended on the laser crystal size, on whether the radiation was focussed, defocussed or unfocussed. Power densities far in excess of 1 gigawatt per cm^2 could be attained. However, air-breakdown near the focal point must be considered in arriving at actual power densities attained. In their studies, no attempt was made to control laser spiking, polarization of the output beam or pulse shape. Pulse repeti-

tion frequencies ranged from more than 1 per second to one exposure per week for several weeks. Duration of exposure to radiation from continuous sources ranged from the order of one second to one hour. Effects of focussed, unfocussed and defocussed radiation were compared. Beam divergence was taken into account (in the investigator's laboratories, but not in field studies), by placing the animals at a fixed distance from the lenses or the laser, or utilising a system of lenses and stops. Intensity variations through the beam cross section were not controlled.

Studies on Skin and Subcutaneous Tissue

The initial studies by Fine, Klein et al (1-5,7,8) were exploratory and concerned primarily with the effects of laser irradiation of the skin, mucous membranes and underlying structures in hamsters (1-3), mice (5,7,8), and rabbits (7,11,16). Although emphasis was placed on the skin as a primary site of interaction in the intact organism, damage to deep seated viscera was observed in hamsters and mice (1-3,5,7,11,16). It furthermore was apparent from these earliest reports (1-3,5) that in addition to heat, other factors had to be considered in understanding the mechanism of the interactions of laser radiation with intact mammalian organisms. The majority of the studies were carried out on small rodents, primarily mice and hamsters. The reasons for conducting studies in small animals were that they represented "scaled-down" model systems, suitable for studying effects of laser radiation which would not have been

demonstrated in larger animals at the relatively low levels of energy and power available at that time.

It should be noted that direct extrapolation of the data obtained in small animals irradiated at low energy and power levels to prediction of specific effects in larger animals at higher intensity of radiation is not indicated. The general principles, however, would be applicable and therefore permit orientation towards those aspects which require further exploration as the investigative conditions are changed.

More definitive studies were continued in small animals not only for practical considerations of space and ease of handling large numbers, but primarily because of the wide range of controlled biological characteristics provided by inbred strains, which are not available in larger species. A number of significant observations were thus made and could be studied in further detail, which could not have been otherwise obtained. These observations included the demonstration of severe, disabling or fatal deep seated injury in the presence of mild superficial lesions (1,4,5,7,11,13,15-19,21-27 following laser irradiation of the intact animals.

At energy levels exceeding 10 joules and pulse durations of the order of 1 millisecond in the region of 6943 A and 10600 A, respectively, the interaction of the radiation with the surface of the animal was associated with a plume of back-scattered material and radiation from the area of impact (1,7,11,13,16).

This phenomenon does not result from electrocautery or other sources of heat, at low power levels. There is no reason to believe that an incoherent source at the same energy and power levels would not produce a plume, similar on gross observations. However, the fine structure of the plume would be expected to differ with radiation obtained from various sources and with the properties of the target.

The skin lesions following exposure to 10-50 joules per pulse at 6943 Å and 10600 Å, focussed or unfocussed, delivered non-Q-switched to the abdominal wall sequentially went through the stages of vesiculation with surrounding inflammation, exudation, crusting, and recovery. Similar skin lesions were obtained when other anatomical sites were irradiated. However, despite the relative mildness of the skin lesions, more than 75% of the animals died within 24 hours if the shaved frontal area or other parts of the head was irradiated at energy levels of 50 joules per pulse (4,11,16). More than 90% of animals survived if the abdomen, chest or pelvis was irradiated at these energy levels. Following irradiation of these areas, however, severe lesions were found deep to minor skin lesions.

The deeper lesions consisted of hemorrhage and edema in the body wall (abdominal, pelvic or chest wall) (16) and the viscera (16) (i.e., intestine, stomach, kidney, spleen and lungs). Between the injured areas of the skin and the severe

lesions in the underlying viscera, however, intervening layers appeared much less severly affected. Lack of free blood in the peritoneal or pleural cavity further indicated that some integrity of the peritoneal or pleural layer had been retained despite extensive hemorrhages and other severe injuries to adjacent structures. These gross observations were further substantiated by the histological findings of alternating injured and uninjured layers of tissues in the path of the radiation (1,7,16). As the energy level per pulse was increased, differential effects on successive layers of tissue became less marked. (11) At higher energy levels, therefore, which would be required to produce deepseated effects in larger animals, the differing reactions of various tissues may be obscured by the intensity of the overall reaction.

Microscopic findings following exposure at 10-50 joules (10-50 joules/cm^2) included sharp delineation between nonviable and viable cells (16). In the skin (1) the sharpness of the boundary was indicated by the close proximity of depigmented hair follicles to pigment containing follicles, and of pigment-free granules to pigment containing granules within the same follicle. Melanin granules in the hair follicles were displaced or scattered. The gross architecture of the hair follicle was retained. Microscopic examination (7,16,25) further showed flattening and elongation of cells and nuclei, as well as swelling and alteration of staining of collagen. Fat cells showed vacuolation. Hemorrhage was present in the deeper tissues, particularly in the underlying muscle.

The microscopic changes of laser-induced skin lesions differ from those produced by the application of a source of heat to the surface. (1,11,16,25) The differential effects produced by laser radiation with different tissue components were considerable more marked than those due to the application of low power conductive heat. The difference between lesions produced by conductive heat (or electrocautery) and laser radiation, respectively, suggested that the critical factors might be associated with the short pulse duration, high peak power and peak power density, as well as thermal properties and diathermancy of the tissue. Phase transformations and secondary interactions due to changes in wavelength or emission of particulate matter also require consideration in this respect.(11, 16, 17) In discussing their findings, Fine et al (1) suggested that for purpose of analysis, the energy could be regarded as being delivered in the form of an impulse.

The relative mildness of the superficial skin lesions did not correlate with the marked severity of the associated deeper lesions following laser irradiation, (1, 16) while lesions produced by conductive heat and electrocautery showed relative uniformity of the degree of damage in the various tissues to which it was applied, with some attenuation with depth. The observations on the disparity between the degrees of injury produced in different tissues by laser radiation were subsequently demonstrated and extended by studies at higher energy (11,16) and peak power levels (5) and at relatively high pulse repetition frequencies (16). The authors report the various levels of total energy, but do not indicate the relationship between the energy used and the specific type of effect observed. Studies on tissue reflectivity, which may be pertinent, were not discussed.

At higher total energy (8,11,15) the severity of the superficial as well as of the deeper lesions was increased. The relation between severity of injury and energy delivered, however, did not appear to be linear. Over wide ranges in the energy of the radiation at both 6943 A and 10600 A relatively minor skin injuries were associated with extensive lesions of the internal structures. This statement is made without describing the experimental observations on which it is presumed to be based.

Goldman et al (29) studied the effects of single pulses of laser radiation at 6943 A at energy levels apparently of 0.34 joules (pulse duration approximately 1 millisecond) on the normal skin of rabbits. At these energy levels no lesions were evident in the albino rabbit. In the skin of the ear of black rabbits complete tissue destruction was produced. Sections of the ear taken 1 hour after exposure to the laser beam showed a deep fissured area through epidermis, dermis and into the cartilage. The dermis showed compression and increased density of collagen with a moderately thick border of neutrophilic infiltration surrounding the irradiated areas. The adjacent dermis showed edema and a diffuse infiltration of lymphocytes and histiocytes. The epidermis adjacent to the impact area showed edema and loss of melanin. From the description given it is difficult to determine the energy levels at which these studies were carried out. It appears that the energy per pulse did not exceed 0.34 joules per pulse. Since unfocussed radiation at this energy density level (from an 1/4" diameter crystal)

would be unlikely to have produced the results described above (i.e. "through and through tissue destruction") it is probable that the beam was focused. The pathological changes in a one hour specimen are described. Further information would be desirable regarding the variations in different animals, and whether the skin was shaved or depilated. The gross or microscopic studies of the lesion immediately following irradiation and at intervals thereafter would be of further interest. The description of the one hour specimen deals with the microscopic appearance of the lesion. The statement "through and through destruction" describes one aspect of the gross appearance which appears to indicate that marked changes were present. In the description of the microscopic examination the changes in the blood vessels or the cartilage and perichondrium would be of interest. These early observations (1962) appear to be preliminary to more complete studies.

Welwig et al (30) reported detailed and careful histological studies on laser induced changes of structure and enzyme activities in the skin of pigs over a range of energy levels. Irradiation was carried out at levels of 1 to 40 joules at a pulse duration of the order of 1.5 millisecond. Energy at each exposure was monitored by beam splitting devices, the fraction

being measured calorimetrically. The problems associated with the use of beam splitters for the accurate measurement of energy will be discussed in a separate section of this report. The relative non-uniformity of the energy distribution within the beam crossection was considered, but of course was not quantitated. However, the general pattern for any specific rod was apparently quite constant. Lasing pattern inversions due to imaging lens systems was recognized. Although the anatomical location of the irradiation site is not stated, it is assumed that equivalent areas were used for comparision, with due consideration for the variation in the characteristics of the skin and underlying tissues, which may affect the interaction. It would be particularly pertinent in comparative studies to consider variations in the depths of the keratin and epidermal layers and in the number and types of epidermal appendages. Although the number of specimens per energy density level is not stated, the description is sufficiently detailed to assure that it is representative, of a number of specimens, and that the usual variability of biological systems has been considered.

Specimens obtained at varying time intervals were studied with hematoxylin and eosin-stained sections and by special stains. The special stains employed were elastic Van Gieson's; colloidal iron stain of Hale for acid mucopolysaccharides (AMP) modified by Pinehart and Abul-Haj, with and without hyaluronidase digestion; Gomori's aldehyde fuchsin; Snook's reticulum; and periodic acid-Schiff (PAS) reaction with and without diastase digestion.

For the demonstration of enzyme activity biopsies were obtained from exposed and unexposed areas 30 min and 24 hr after exposure. Apparently no specimens were obtained immediately after irradiation, although thermally induced denaturation of enzymes would have been expected to have been present immediately. A 30 minute interval could be sufficient to permit loss of enzyme activity due to secondary causes, such as loss of blood supply, release of proteolytic enzymes or accumulation of metabolic acids due to primary alteration of specific cellular components. In each substrate solution, sections from unexposed areas were incubated simultaneously in the same substrate solution with sections obtained from exposed areas. Biopsies were obtained from exposed areas at varying intervals including 30 min, 3.5 hr, 3 days, and 11 days. The histological and histochemical descriptions are of an obviously high scientific caliber and are therefore presented below with minimal modification, but with some discussion.

At an elapsed interval of 30 min, at an energy of 9 joules and an image diameter of 2 mm, the epidermis showed a minute epidermal vesicle. The basal cells were stretched and thin and some remained attached to the cutis. In the epidermis above the vesicle, the nuclei tended to be pyknotic and superficially there was parakeratosis. It would be of interest to know whether the authors consider the observed vesiculation to be intra-epidermal or subepidermal. Contiguous with the lateral margins of the vesicle, the epidermal cells, particularly in the lower epidermis, were vacuolated. In some areas, though the upper epidermal cells

showed a vesicular and vacuolated cytoplasm, the epidermal changes were not sharply defined laterally, probably since focussed radiation was used and examination was at 30 minutes rather than immediate. In the corium immediately subjacent to the vesicle, some collagen fibers exhibited a blue tinge and the superficial capillaries were collapsed. A few subjacent capillaries contained thrombi. Beginning at the margins of the vesicle and extending laterally, the capillaries in the upper corium were dilated and hyperemic associated with clear spaces between the collagen fibers. The clear spaces observed by the authors may have been due to local vaporization of tissue fluid within spaces confined by the fibers.

The entire upper corium was moderately positive in the colloidal iron preparation and showed loss of staining in sections preincubated with hyaluronidase, indicating the presence of hyaluronic acid. The hyaluronic acid was not decreased beneath the vesicle.

At an elapsed interval of 3.5 hr. at an energy of 10 joules and an image diameter of 2 mm, the epidermis showed a subepidermal vesicle partially filled with pale pink homogenous material. (Since the laser and lens characteristics are specified, the expected beam divergence within the tissue can be calculated, if the relative refractive index of the tissue and the laser beam divergence is considered). The basal epidermal cells in the roof of the vesicle were elongated and the cells above the basal layer showed a pale cytoplasm, with nuclei varying from pyknotic to ballooned and empty. The superior layer appeared parakeratotic. At the lateral margins of the vesicle, the epidermis showed

vacuolization of cells, especially in the deeper layers. A few of the vacuolated cells contained PAS-positive material (glycogen), but most of the vacuoles did not show a specific content by special stains. The basal cells exhibited less of a picket-fence orientation and tended to be polygonal. Nuclei in this area were enlarged and sometimes had pale, granular chromatin and one or more distinct nucleoli. The granular cell layer was decreased, and there was parakeratosis. Mitotic figures were rarely noted in the basal layer. Laterally, the altered epithelium blended with the normal epithelium. The base of the vesicle was covered with a basement membrane. The subjacent corium in one area showed bluish-pink homogenous collagen fibers, but elsewhere the fibers were normal red. In the bluish-pink areas, the capillaries were not apparent, but were present and dilated, particularly beneath the lateral angles of the vesicle. Capillaries were also dilated and hyperemic in the upper corium lateral to the vesicle. Many capillaries in the upper corium were surrounded by polymorphonuclear leukocytes. No appreciable change was noted in the amount of hyaluronic acid in the upper corium beneath the vesicle. Elastic tissue and reticulum did not appear altered.

At an elapsed interval of 24 hr, at an energy of 10 joules and an image diameter of 2 mm. the epidermis showed an intraepidermal vesicle and pustule. The roof of the vesicle appeared to be

comprised of most of the original epidermis, and many of the epidermal cells were represented by shadowy out-lines. Nuclei exhibited various stages of degeneration. At the base of the roof of the pustule there was karyorrhexis of nuclei and leukocytes. The vesicle also contained erythrocytes. The base of the vesicle showed one or more incomplete layers of epidermal cells extending centrally from the lateral margins of the vesicle. The authors do not indicate whether the epidermal cells at the base of the vesicle represent regenerating rather than residual epithelium, although the former is suggested to the reviewers by the statement that extension from the lateral margin was apparent. In one segment the epithelial cells at the base of the vesicle were obliterated by leukocytes. This, however, would suggest to the reviewers, that the epidermal layers at the base of the vesicle were residual rather than regenerating cells, (possibly damaged by micro-organisms) which had undergone progressive degeneration prior to leucocyte infiltration. Elsewhere the cells at the base of the vesicle varied in size and frequently contained clear vacuoles. The cells at the base of the vesicle contained an increased amount of glycogen, and there was an increased amount of colloid iron positive material among the epidermal cells and apparently within the cyto-plasm in fine granules. Some of this material was not removed by digestion with hyaluronidase. Nuclei also remained blue.

An area of the superficial corium stained a bluish pink. Except in this area, capillaries were dilated and erythrocytes and scattered leukocytes infiltrated the corium. The colloidal iron preparation showed a decreased amount of acid mucopolysaccharide in the superficial corium beneath the vesicle.

At an elapsed interval of 3 days, with an energy of 8.4 joules and an image diameter of 2 mm, the epidermis showed a scale of parakeratotic cells and degenerated epidermal cells within which were a few degenerated leukocytes. This scale was loosely attached to the underlying epidermis, which appeared to be of average thickness but showed epidermal cells with pale cytoplasm and slight distortion in arrangement. The basal layer did not show a picket configuration and at the lateral margins of the area some cells displayed a vacuolated cytoplasm. One area in the upper corium beneath the scale exhibited bluish-pink collagen fibers, and there was extravasation of erythrocytes. Capillaries were dilated and hyperemic, and a few leukocytes were in the surrounding stroma.

At an elapsed interval of 11 days, with an energy of 9.6 joules and an image diameter of 2 mm, no residual vesicle or altered epidermis could be identified. The subjacent corium showed capillary hyperemia and a few leukocytes within the stroma.

One specimen of skin was exposed to a soldering iron at 250 W for 1 sec and biopsied after an interval of 30 min. Most of the epidermis was charred and desquamated. The underlying superficial corium

stained various shades of red in the hematoxylin and eosin preparations, and appeared coagulated. There was no capillary, leukocytic, or fibroblastic response. No acid mucopolysaccharide was identified in the upper corium. The epidermis at the margin of the charred area showed colloidal iron positive material within several epithelial cells but none in the adjacent corium. Elastica and reticulum could not be identified in the upper corium. It is of interest to note that in the specimen exposed to heat from a soldering iron, the reaction differed markedly from the reaction to laser radiation. It should be noted that it is difficult to quantitate the thermal energy involved, and to compare the radiation from the laser with that from the soldering iron. It is apparent that the authors did not intent to compare specifically the effects of a soldering iron with that of laser radiation.

In the normal pig skin succinic dehydrogenase activity is most intense in the basal cell layer with less activity in the more superficial epidermal cells. DPN-diaphorase, and lactic dehydrogenase have strong activities in the epidermis with much less accentuation in the basal layer than with succinic dehydrogenase. The isocitric and glucose 6-phosphate dehydrogenases are confined, for the most part, to the sebaceous glands and sweat glands.

No alterations of enzyme activity or cell structure were found at either interval with an exposure of 2 joules. An exposure of

3 joules focused on an area 2 mm. in diameter of skin surface resulted in a marked decrease of SDH, LDH, DPN-diaphorase, and TPN-diaphorase activities at both the 30 min and 24 hr intervals that was confined to the area of anatomically altered epidermal cells and adjacent structurally normal cells. The same energy focuses on an area 4 mm in diameter resulted in a broader area of decreased enzyme activity; however, the decrease in activity was not apparent until the 24 hr interval and no structural changes were found. The delay in loss of heat labile enzymatic activities would indicate to the reviewers that this was due to secondary effects, rather than to primary thermal effects resulting in heat denaturation, which should have been apparent much earlier.

An exposure of 11-12 joules resulted in a loss of enzyme activity in the epidermis for SDH, LDH, DPN-diaphorase, and TPN-diaphorase at both time intervals. When the area of exposure was 2mm in diameter, the decreased enzyme activity was confined to a small area of the epidermis with an abrupt transition between the adjacent area showing relatively strong activity and the zone of diminished enzyme activity. When the area of exposure was 4 mm in diameter there was a focal loss of SDH, LDH, DPN-diaphorase, and TPN-diaphorase activities in the epidermis. Zones of structurally normal cells having strong enzyme activity were interspersed with the areas of decreased or absent activity. Alterations of IDH and G-6P-d activities in adnexal structures were not encountered with any of the energies used up to and including 12 joules.

The authors (30) believe the laser lesions are comparable to thermal injury in pig skin. This comparison and the equations quoted will be discussed by the reviewers in another section of this report.

The authors suggest (30) that the laser induced lesions in the skin can be explained without bringing other physical effects into play. The magnitude of the predicted thermal effects are further considered as limiting the detectability of other physical or biologic effects. This conclusion is based on studies at the energy and power levels used in the specific system examined (pigskin). At other energy and power levels, or in other biological systems (i.e. frontal area of the head of the mouse) other physical and biological effects become detectable, which cannot be explained on the basis of the usual thermal factors without change of phase. Consequently, it would not be possible to extend the thermal hypothesis (even if correct) applied to these studies, to general conclusions which would be applicable to biological effects of laser radiation under varied experimental conditions.

Detailed pathological studies of the effects of laser radiation on the skin and underlying structures of the abdominal wall were reported by Laor, Simpson, Klein and Fine (25). Their studies are included in full, since the publication which is currently in press will not be available for some time.

Most of the studies (described below) were carried out with single pulses obtained from ruby crystal lasers (9.5 mm. rod diameter) operating at a wave length of 6943 A, at energy levels of 3-100 joules per pulse and at pulse durations of the order of 1 millisecond. Correspondingly larger ruby laser units were used at higher energy levels. Some studies were carried out with neodymium in glass units operating at a wave length of 10,600 Å and at energy outputs ranging from 300 to 900 joules per pulse. The radiation was unfocussed or focussed to a spot size of 1-2 mm. at the skin surface by a simple lens system. This spot size was determined by transillumination through the crystal, and is not necessarily the spot size attained with the laser radiation. Swiss (white) and C 57 (black) mice of various ages and both sexes were used. The animals were anesthetized, the hair clipped and the area depilated chemically prior to irradiation. The animal was then mounted on an optical bench to facilitate alignment with the lens system and the direction of radiation. A light

beam was projected through the axis of the laser crystal to assist in localising the irradiation site on the mouse. The laser to target distance was measured.

Animals were sacrificed immediately following exposure and at intervals thereafter ranging from 1 hour to 26 days. There was a minimum of 4 animals in each group (Table I: Groups 1, 4, 5). The animals were autopsied, except for 66 irradiated mice and non-irradiated controls which were kept for life-time follow up. Some of the observations (Table I: Groups 3, 7, 9) could not be further pursued as they were obtained from exploratory studies carried out with laser devices at early prototype stages of development. These devices were primarily intended for developmental uses other than biomedical investigations. Their availability was therefore limited. Despite the small number of animals in these groups, the information not otherwise available was pertinent and is included.

Since data on thermal injury in mice was not readily available in the literature, 28 lesions were induced by manor bipolar electrocoagulation for compasison with the effects of laser radiation. The cautery needle, through the skin to the underlying tissue, was applied manually for the minimum possible time. Ten other lesions were induced by a steel rod which had been kept in boiling

water prior to application or by pipetting of boiling water onto the surface of the skin. An additional group of 132 mice were exposed to a mercury lamp. (Water cooled GE-AH-6 1000 watt high pressure mercury lamp using the optical system of a Scopicon projector unit).

Skin lesions were visually examined and photographed periodically. Skin and internal lesions were recorded at autopsy. Material for histological examination was fixed immediately in 10% buffered formalin, embedded in parafin and stained with hematoxylin and eosin. Selected slides were stained with Fontana stain for melanin, reticulin-trichrome and Mayers hematoxylin. Frozen sections were cut from some of the material and stained with Oil red O. Serial sections were not made routinely, but multiple levels were sectioned in the absence of a gross lesion.

One hundred and ninety mice were included in studies on unfocussed irradiation. Immediately following irradiation a slight discoloration was seen over an area of up to 10 mm. in diameter, corresponding to the laser rod diameter, in almost all mice that had been exposed to 70-100 joules. Below 70 joules, immediate gross lesions were seen in a decreasing proportion of mice. No immediate lesions were recognized on gross observation below 20 joules. From 300-900 joules, the lesions measured about 15 mm. in diameter, corresponding to the larger diameter of the rod, and consisted of a paler outer zone surrounding a reddish-brown area with central destruction of the epidermis. The lesions were similar at different anatomical sites except that rupture of the skin

of the forehead with exposure of the skull occurred in three animals receiving approximately 860 joules at 10,6000 Å to this area.

After reflection of the skin, a few petechiae were seen occasionally on the deep surface following irradiation at 20 joules. These were present more frequently as the energy was increased to 70 joules. Above 70 joules, diffuse hemorrhages occurred. Above 300 joules, hemorrhage was absent from the central 3-4 mm. of the lesion on the inner surface of the reflected skin. Beyond this area, there was a circular region of necrosis 5 mm. in width, separated from normal tissue by a hemorrhagic ring 1 mm. in width.

Diffuse hemorrhages were occasionally seen in the abdominal and chest wall muscles at energy levels of less than 70 joules and were almost present above this energy level. The hemorrhages involved areas up to 20 mm. in diameter. Even the more severe hemorrhages did not result in rupture of the muscle sheath. At energy levels above 300 joules the lesion of the muscular layer coincided with corresponding zones on the surface of the skin. The degree of hemorrhage was not constant at a given energy level. Lesions in the underlying viscera, especially the liver, were sometimes produced at 20 joules and were almost always present at 30 joules and above. Injury to the intestines and the brain were usually responsible for death occurring within the first few days after irradiation.

One day after irradiation, the skin lesion appeared as a patchy, superficial, reddish-brown, dry area measuring up to 10 mm. in diameter. Two days later, a firmly adherent scab, approximately 1 mm. thick, covered the entire surface in the majority of cases after 70-100 joules. Below this level, the lesions were less severe. Above 70 joules, the skin lesion was adherent to a larger area of whitish discoloration and loss of transparency in the superficial muscle sheath over and close to the site of the hemorrhage.

Ninety-two mice were studied following focussed irradiation. A greyish-white umbilicated skin lesion, 1-2 mm. in diameter, was present immediately after exposure to energy levels ranging from 10-100 joules. The severity of the resulting lesion was proportional to the delivered energy. Exposure to energies above 300 joules resulted in umbilicated lesions, 3-4 mm. in diameter, surrounded by a pale circular zone. A reddish discoloration was present within the central crater. Lesions from 300-900 joules were indistinguishable by gross observation. The effects on the deeper tissue at energy levels up to 100 joules were similar in appearance and size to those observed after unfocussed irradiation. Above 300 joules the effects on the deeper tissues extended over a smaller area than in the lesion following the corresponding amount of unfocussed radiation and were better tolerated by the animal.

The healing process was similar to that following unfocussed irradiation. The crust in the central crater at all energy levels

separated and the umbilicated lesion flattened within 4-5 days.

Following recovery, the majority of mice being kept for long-term evaluation showed no delayed visible abnormalities. The lesions healed within 2-3 weeks after irradiation, depending on the size and severity of the lesion, and left no macroscopic scar. In several black mice scattered throughout the groups, temporary failure of melanogenesis during the recovery period was observed, but the following hair generation showed no abnormal pigmentation. In one mouse a chronic ulcer is present one year following irradiation. Some differences other than depigmentation were found between the reaction to the laser irradiation in black and white mice.

Twenty-two of the sixty-six mice (Table I: Group 2,5_) which were kept for life-time follow up died at intervals ranging from 3 weeks to 16 months following irradiation. The remaining forty-four animals are still under observation.

Like the gross appearances, the immediate microscopic changes in the skin following unfocussed irradiation are generally patch at energy levels below 100 joules. There is considerable variation in the skin lesions, but not in the subcutaneous lesions, among mice apparently exposed under the same conditions.

In the epidermis of animals sacrificed immediately or within a few hours, the minimum change is a shrinking and flattening of epidermal cells and of their nuclei in a zone 1-2 mm. in diameter. Nuclei are

hyperchromatic and cell borders indistinct. In some mice there is a slight separation of the epidermis from the underlying dermis. A narrow zone of basal cells with small, hyperchromatic nuclei and a perinuclear vacuole, is frequently present around the flattened cells. These changes are rarely recognizable following irradiation at energy levels less than 20 joules. Above 70 joules multiple foci of flattening, separated by 2-3 mm. of normal epidermis are present within an area of 10 mm. in diameter. More severe changes, occasionally seen below 70 joules, occur with increasing frequency above this level. The cellular changes are similar but the cells are twisted and distorted and the epidermis may be increased in thickness over an area 3-4 mm. in diameter in the center of the lesion. Small spaces are present within and beneath the epidermis. The hair follicles are involved to a varying extent. If the lesion extends down to the hair roots and melanin granules are present, the granules are scattered with the hair and hair root and sometimes into the surrounding tissue. Above 300 joules these changes extend for 0.7-1.0 mm and the epidermis is totally separated in the center of the lesion.

In the dermal connective tissue, minute foci of swelling and basophilia of the collagen fibers are sometimes recognizable beneath areas of flattened epidermis. The amount of visible change in the collagen, which is rate below 30 joules, increases as the energy level is increased. Below 70 joules, alterations are generally restricted

to the outer one-third of the dermis with an occasional deep zone of similar change surrounding hair roots. Between 70 and 100 joules, a 3-4 mm. zone of coagulation involving the full thickness of the skin is occasionally present. This is associated with the most severe of the epidermal changes described above. Following irradiation at energy levels in excess of 300 joules, coagulation is consistently present in an area 7-10 mm. in diameter through the full thickness of the skin.

One of the most consistent findings, sometimes present without other evidence of damage in the skin, is swelling of the adipose cells in the deeper dermis, so that they appear abnormally large in paraffin sections. Following irradiation in excess of 300 joules, larger spaces appear, suggesting breakdown of the cell walls. Fat can be demonstrated in some, but not all, of these spaces in frozen sections.

Injury to dermal capillaries with leakage of red cells accounts for the petechiae in the skin at low energy levels. Rupture of small arteries and veins is responsible for the larger hemorrhages in the skin and underlying muscle present following irradiation at 40 joules or more. Necrosis of the vessel wall is responsible for the larger hemorrhages in the skin and underlying muscle present following 40 joules or more. Necrosis of the vessel wall is associated with small perforations. At the edge of the perforation the cells are distorted and elongated and may project outwards from the lumen. The nuclei are similarly distorted. At the site of rupture the red cells are usually fused into an hour-glass shaped mass which is partly outside the vessel.

The cell outlines are not usually recognizable, but sometimes elongation of neighboring corpuscles is seen. Multiple perforations about 2 mm. apart may be present. In the central coagulation zone of lesions following 300 joules or more the vessels are recognizable only as an irregular mass of basophilic material associated with a smaller mass staining orange-red with eosin representing the blood. This corresponds to the area in which no hemorrhages are seen grossly.

No muscle damage was seen following unfocussed radiation at an energy level of 7 joules, but it might have been demonstrated had the material been serially sectioned. Above 10 joules, muscle lesions of increasing size are present and at 40 joules the full thickness of the abdominal and chest wall is involved at the center of a 10 mm. zone of damaged muscle. Above this energy level, the extent and severity of the muscle lesion increases, reaching 12-14 mm. in diameter at 860 joules. Vacuolation and fragmentation of cytoplasm is seen in the center of the milder lesions and in the outer zone of the more severe lesions. Shrinking and more intense staining of cells and nuclei occur in the center of the lesions following irradiation at higher energy levels. Above 300 joules this zone is 7 mm. across and involves the full thickness of the muscle. Collagen changes similar to those described in the dermis are present in the sheaths and septa. Hemorrhages are more extensive than in the skin, and the vascular perforations tend to be larger. In some sections where the skin and abdominal wall were removed

together, attachment of the platysma by loose connective tissue to the sheath of the underlying muscles appears to be lost. Occasionally platysma cells are abnormally elongated or apparently absent in the center of the lesion.

The immediate skin lesion following focussed irradiation is, as expected, smaller but more severe than that following the comparable unfocussed beam. A zone of flattened epidermis is recognizable following irradiation at 7 joules. Between 40 and 70 joules, total epidermal necrosis and coagulation of the full thickness of the dermis becomes increasingly common. Between 70 and 100 joules, the lesion is 3-4 mm. in diameter, with a central area of total coagulation 2-3 mm. across. In a specimen following irradiation at over 300 joules, the area of total coagulation of the skin was about 3 mm. in diameter. Other changes were not satisfactorily identified because of post-mortem changes.

In general, the deeper lesions resemble those produced by the corresponding unfocussed beams. A minute lesion in the subcutaneous tissue and muscle was recognized at 7 joules. No lesions were found at this level after unfocussed irradiation.

The healing process is similar in lesions produced by focussed and unfocussed irradiation with comparable tissue damage. One day after laser irradiation, healing is already occuring. In the mildest lesions the only evidence of laser injury may be hyperplastic epidermal cells with a thin layer of dead cells on the surface. It is probable

that these lesions are not recognizable microscopically immediately following irradiation as changes present at 7-10 joules. At higher energies the lesion extends over a larger area than in comparable specimens examined immediately following irradiation at 7-10 joules. There is a slight diffuse polymorphonuclear infiltration in the dermis associated with edema and vascular dilatation in the milder lesions such as occur at 20-40 joules of unfocussed irradiation. Necrosis of small blood vessels in the deeper dermis with perivascular inflammatory cells is present in more severe lesions. A heavier inflammatory infiltrate surrounds areas of coagulated dermis. Edema fluid and a scanty cellular exudate may be present between the platysma and underlying abdominal muscle.

Three days following irradiation, nuclear pyknosis and karyolysis is marked in affected skin and muscle. Polymorphonuclear infiltration is less marked at the epidermal level, but may still be seen around deeper necrotic tissue. There are more macrophages, especially in adipose tissue and muscle. Hematoyphil material, suggestive of calcium deposits, is often present in these tissues. Vessels with obviously necrotic walls contain thrombi. Degeneration is now recognizable in nerves between the muscle layers. Swollen mesothelial cells and some fibrinous exudate are seen over the peritoneal surface in some cases. Scabs have formed and are partially separated. Epithelial regeneration is active at the edges of the lesion and from the necks of the hair follicles.

After five days, if an ulcer is still present, the base is granulation tissue. Epithelial regeneration is very active with abnormally thick epidermis and large adnexa. In the deeper tissue many macrophages are

present around dead muscle and collagen. At 70-100 joules, the surface lesion is now about 8 mm. in width and the damaged muscle zone is about 12 mm. across, of which up to 9 mm. is totally necrotic. Fibroblasts are present in necrotic vessel walls, in the blood clot within and around the vessels, and in adjacent dead nerves. Toward the edge of the lesion red cells are seen within vessels with an apparently necrotic wall. Fresh hemorrhage into the tissue is common in these areas. Nerves adjacent to these vessels usually appear normal. At the edges of the dead muscle the surviving fibers are narrow with basophilic cytoplasm and central hyperchromatic nuclei.

It appears on microscopic examination that the line of separation is frequently located deeper in the dermis during the phase of hair pigment formation in black mice, than during the rest of the hair cycle. The lesions in the white mice also appear to be deeper at a comparable phase of the hair cycle.

In a few lesions studied later than 5 days, healing appears to have progressed to fibrous scars as would be expected for any comparable injury. Occasionally hypertrophic epithelial cells, especially in the adnexa, have been seen at the site after several months.

Many of the pathological changes found following laser irradiation are also seen in various types of burns. However, the vascular perforations and scattering of melanin granules appear to be exceptions. The damage to deeper tissue following laser irradiation is proportionately more severe than in thermal burns with comparable degrees of surface injury. This is not a species difference since lesions in mice subjected

to thermal burns or to electrocautery during the present experiments closely resemble those in the larger animals used in other reported studies. It is not known whether the differences from previously described thermal injuries are due merely to the uniqueness of the distribution of heat produced by laser radiation or whether other factors such as those associated with phase transformations, charged particle production and wave length alterations are of significance.

In thermal burns there is a temperature gradient from the surface into the deeper tissues. In the pig, Moritz (31) found that it required a temperature of 45° C for 3 hours or of 100° C for 0.1 second to obtain complete destruction of the epidermis. Under these conditions, damage to a 2 mm. depth was quite severe at 45° C, while there was no damage at 100° C. In their studies on high intensity radiant energy burns in the rat, Sheline and co-workers (32) found a correlation between the amount and depth of tissue injury and the extent of surface damage. They also found that the severity of surface burns was increased, if the energy was delivered within a shorter period (33). With the laser beam, which is applied for approximately 1 millisecond, severe damage to internal viscera at a depth of 2 mm. or more was produced, without total epidermal necrosis over a corresponding area and without uniform damage to the intervening tissues. This is partially due to the optical properties of the tissues which differ in their capacity to transmit or absorb radiation at various wave lengths. These differences affect the initial absorption of energy and consequent temperature distribution within the tissues.

Differences between laser-induced injury and other types of burns are also dependent on the extremely rapid rise of temperature within the

path of the beam. The extreme distortion of cells and nuclei surrounding apparently empty spaces seen in the laser lesions also occurs in burns produced by electrocautery immediately adjacent to the needle tract. It seems probable that these lesions are due to extremely rapid vapor formation in the tissues from the very rapid rise in temperature.

The short pulse duration, the thermal time constants of the tissue and the severe effect of the laser radiation on blood vessels also limit rapid lateral heat flow. This is in contrast to thermal burns where at lower temperatures and longer exposures blood flow was found to influence the dissipation of heat in the deeper tissue. Direct measurements of temperature distribution in biological systems produced by the laser beam is difficult since the thermal detectors, such as thermocouples, would be within the path of the radiation, and would therefore, be directly affected by the radiation.

Heat production is believed to play an important part in injury from ultrasonic irradiation. The skin burn produced by ultrasonic radiation is similar to other burns. The deeper injury from this type of radiation differs from that following laser irradiation, since the reflection of the sound waves at tissue interfaces leads to a different distribution of lesions. After ultrasonic irradiation, the blood vessels are frequently less affected than the surrounding tissue in contrast to the severe vascular lesions observed following laser irradiation. Hypersonic frequencies have been reported following laser irradiation of solids and liquids. The production of ultrasonic and hypersonic

frequencies, therefore, requires further assessment in regard to tissue injury.

Variation in the effects of laser radiation due to the relative absorptivity of the specific wave length at the impact site has been further investigated in vitro, in melanomas, and in intraocular lesions. It has been shown that the size of the lesion in the rabbit eyes depends on the intensity of the laser beam and the pigment density of the retina and choroid. Frequently, melanin pigment is displaced from the choroid following laser irradiation of the eye. This resembles the scattering of melanin from pigmented hair roots. Painting the skin of the mouse with dyes which absorb the radiation, results in a more widespread and severe superficial lesion with less damage in the deeper tissues. The relative importance of pigments such as hemoglobin, myoglobin and lipofuschin has received less attention than that of melanin. In the experiments reported here, lesions in the abdominal muscle and liver were common even in the presence of minimal skin damage. At energy levels as low as 20-30 joules, a zone of necrosis 2-3 mm. in diameter and 1 mm. deep was seen in the liver. At higher energy levels the necrotic lesion was larger. The possibility exists that the pigments present in these organs are responsible for the apparently disproportionate damage, which would be enhanced in the liver by its vascularity and absorptivity at the wave length used. The presence of microscopic evidence of injury immediately after irradiation minimized secondary effects from vascular damage as the cause of the immediate lesions.

As hair growth was not always controlled in these experiments, the number of animals observed at each stage of the hair cycle is insufficient to reach a definite conclusion concerning the importance of this

factor. Transmission of heat down the hair shaft or mechanical factors associated with stretching of the skin, as discussed below, may be responsible for the increased damage at the level of the hair bulb, apparently present in white as well as pigmented mice. However, the hair cycle is accompanied by changes which increase the susceptibility of the skin to ultrasonic radiation (34) and these changes may also influence the response to laser irradiation. Variations in age, sex and nutrition as well as the hair cycle, which may modify the reaction to the laser beam, are currently under study.

Blood vessels in the skin and underlying muscles seem particularly susceptible to damage from laser irradiation. The perforations of blood vessels described here have not been reported following other burns. The perforations may be due to increased intravascular pressure from high absorption of the energy by the red blood cells with consequent vaporization and pressure formation. Other modes of entrapment of energy within the vessels may also be significant. The etiology of vascular perforations in the skin and subcutaneous tissue requires further investigation. Similar lesions do not occur in other organs, for example in the liver, following irradiation at comparable energy levels (unpublished data). Possibly the diameter of the vessels, the method of branching or the supporting tissues are critical. Vascular perforations were seen in irradiated skin flaps, in which an ischemic area resulted, but diffuse hemorrhages were absent. In general, skin damage in an irradiated skin flap after 70-100-joules was not commensurate grossly or microscopically, with that produced by a similar

amount of laser energy applied to the intact animal (unpublished data).

Outward hemispherical dissension of the skin of the mouse was observed by high speed photography during laser irradiation at 40-100 joules. This is accompanied by back-scattering of particles and radiation from the surface of the animal, previously described as the "plume". If the insult is repeated at the same place, similar effects occur but become progressively less pronounced. The rapid stretching of the skin and adjacent structures may be of significance in the laser injury. Although it appears that this ballooning is due to vaporisation within the tissues, the exact levels at which it occurs has not been determined. It is tempting to place it in the subcutaneous tissue. This would account for the apparent stretching and rupture of the platysma and connective tissue bands sometimes seen microscopically. The pressure wave travelling in the opposite direction would stretch the underlying muscle. It is also possible that considerable absorption of energy occurs within the muscle layer resulting in phase transformations, stretching and rupture of the muscle fibres and blood vessels. This stretching would account for the frequency of massive hemorrhages observed in the intact animals but not in skin or abdominal wall flaps. Absorption of energy in the layers between the epidermis and the skull together with the increased resistance to gaseous expansion offered by the rigid skull may be significant in a consideration of outward rupture of the skin following high energy irradiation as well as in the production of intracranial injuries.

Discussion of these experiments must include a consideration of the fairly wide variation in the results with supposedly equal amounts of laser energy. In general, however, the effects on the skin are comparable if considered in terms of energy distribution per unit area. In the case of the focussed beam, some variations are undoubtedly due to the difficulty

of accurate focussing on the surface because of respiratory and other movements. Another factor affecting results obtained with both the focussed and the unfocussed beam is a change in the energy output of the laser. Following several hours of use, the energy per pulse decreased by about 10 per cent. In the early phases of the study, the importance of the beam divergence associated with target to laser source distance was not appreciated. However, this was probably a minor factor in any variations which occurred, as indicated by measurements of beam divergence. The width of an unfocussed laser beam at 40 joules was found to increase from 9 mm. at 50 mm. distance to 14 mm. at 500 mm. distance.

The distribution of energy within the laser beam is not uniform. For example, more energy may be present at the center and the circumference of the beam than in the intervening area. This variation is one of the factors resulting in the patchy skin lesions following unfocussed irradiation. In contrast to the wide variation in the effects of focussed and unfocussed beams on the skin, the deep lesions are remarkably uniform. They occupy a wider zone than the skin lesion if the beam was focussed, partially due to beam divergence and scattering of the radiation, and an equal or smaller zone than that observed superficially following unfocussed irradiation.

The biological effects of laser radiation at a single wave length and within relatively narrow ranges of power and energy vary with the characteristics of the radiation and the anatomical sites. Since the mechanisms of the interactions are only partially understood (17), it would be difficult to extrapolate from these data to anticipate the effects

on other tissues or of radiation of different characteristics. Considerable additional studies are therefore required to understand the basic biological and physical factors taking part in the interaction, particularly for evaluation of the potential hazards (17) and meaningful clinical application of the various types of laser radiation.

Many of the pathological changes produced by ruby and neodymium kilowatt laser irradiation in the skin and underlying muscles of the mouse can also be produced by heat. Because the heat generated following laser irradiation is dependent on the optical and thermal properties of the tissues and on the initial energy distribution within the tissues, the injury does not decrease uniformly along the path of the laser beam. The pigmented tissues are more severely affected than the non-pigmented structures.

The differential absorption and scattering of radiation in association with the extremely rapid rise in temperature within the laser beam path probably accounts for such characteristics lesions as perforations of the blood vessels and scattering of melanin granules. Rapid phase transformations to the vapor phase within relatively rigid boundaries on a macroscopic or microscopic basis may account for some of the observed effects.

Further studies are required as a basis for elucidation of the biological effects, for evaluation of potential hazards and for medical applications of laser radiation.

. Irradiation of the Abdomen and Pelvis.

Fine et al (1) observed visceral lesions in the presence of relatively minor superficial lesions following laser irradiation of intact hamsters and mice. Further studies by these authors (7,11,16) were directed towards a more detailed investigation of effects on deep seated tissues.

In studies on mice at energy levels in the 50 joule range per pulse obtained from a 3/8" diameter ruby laser (7,11,16). the majority of mice survived focused and unfocused irradiation of the anterior abdominal wall. In periodically sacrificed animals, changes in lesions of the underlying viscera (liver, intestine) were followed (16). Despite extensive hemorrhage and severe injuries to adjacent structures, free blood was not present in the peritoneal cavity. Between some injured tissues, there were intervening layers which appeared to have undergone less damage. Superficial skin lesions were well demarcated following focused or unfocused irradiation. Underlying lesions of the liver were also well delineated following both focused and unfocused radiation directed at the overlying intact skin. Intestinal lesions varied in severity and degree of delineation.

The hemorrhages in the deeper tissue appeared to be related to gross vascular damage, necrosis and, occasionally, rupture of the vessel wall. In hepatic lesions a sharp transition from structurally altered to morphologically unchanged tissue was observed. In some cases, parenchymal degeneration extended along the hepatic vessels beyond this line of transition. The general

architecture of liver was preserved for at least 5 days. In the central part of the hepatic lesion, elongation and distortion of cells and nuclei was present. Intense congestion occurred in the sinusoids of the outermost part of the lesion immediately after irradiation, but frank hemorrhage was not observed. Necrosis of the intestinal wall usually involved a part of the circumference, and was frequently associated with hemorrhage into the wall and lumen. More severe intestinal lesions caused perforation and peritonitis which resulted in death. Microscopic lesions were seen in the pancreas, stomach and other abdominal and pelvic organs (16).

As indicated in other sections of this report, the irradiation of the intact animal is accompanied by a sudden hemispherical distension within the abdominal wall which was monitored by high speed photography at 8,000 frames per second (7). It is difficult to determine the extent to which some of the gross and microscopic changes are due to thermal factors or other factors, such as the production of transient phase changes.

At energy levels exceeding 300 joules at 6934 Å, directed at the intact abdominal surface of mice, the effects were more marked than in the 75 joule range (7, 11, 16, 25). Survival time varied from immediate death to death within two days following irradiation. Free blood was present in the peritoneal cavity. Gross and microscopic lesions were present in the kidney, spleen and pancreas as well as in the liver and intestine. At energy

levels exceeding 500 joules, at both 6934 Å and 10600 Å, similar although more severe effects were observed(7, 11, 16).

. Irradiation of the Thorax.

Radiation at energy levels below 100 joules directed at the chest, whether focused or unfocused, did not result in death (7, 11, 16). Energy levels exceeding 300 joules at 6934 Å resulted in immediate death to death within two days (7, 11, 16), depending on the exact location of the impact. On autopsy, gross lesions were present in the lungs and heart. Sharp demarcation between hemorrhagic and relatively normal lung was evident on gross inspection. On microscopic examination, congestion of alveolar walls, frank hemorrhage into alveolar spaces, and almost complete loss of the staining properties of the cells in the alveolar wall was observed. Some of the vessels within the affected zone contained fused blood. At energy levels exceeding 500 joules, at both 6934 Å and 10600 Å, directed at the chest or abdomen of normal mice, survival times varied from immediate to two days post-irradiation. The deeper lesions in the thoracic organs, as in the abdominal viscera at these energy levels were more extensive than at lower radiation energies. In general, the focused beam was better tolerated than the unfocused beam at equal outputs of energy and the extent of the lesion caused by the focused beam was confined to a smaller area.

. Irradiation of Exposed Abdominal, Pelvic, and Thoracic Organs

In view of the marked effects on viscera when intact animals

were irradiated, individual organs were exposed directly to laser radiation (7,16,26). Laser irradiation of exposed organs of white Swiss and black C57 mice was carried out at 6943 Å and at energy levels ranging from 4 to 75 joules. Both unfocused and focused irradiation was employed. The exposed organs included liver, spleen, pancreas, bladder, testes, kidney, stomach, lung, heart and brain. Following irradiation the incision, through which the respective organ(except the brain) had been exposed, was closed. Irradiation of the stomach and intestine at energy levels of 75 joules produced intestinal perforation and death. Irradiation of the other abdominal viscera produced non-fatal lesions which were tolerated by the animals without apparent disability.

The immediate effects of unfocussed radiation at 75 joules per pulse (spot size - 9 mm in diameter) on the liver was a well circumscribed dark red lesion. On sacrifice two days post-irradiation, this lesion appeared as a well circumscribed white area with a narrow red margin. Focused irradiation at 75 joules per pulse (spot size - 3 mm in diameter) resulted in rupture of the capsule, tissue volatilization, and crater formation. Minimal bleeding occurred in some cases, but ceased spontaneously within several minutes. Following unfocused irradiation at energy levels below 12 joules (spot size - 9 mm in diameter) lesions were not evident on gross examination. At energy levels above 18 joules, lesions in the liver were observed macroscopically.

Lesions produced by focused or unfocused irradiation (energy levels of 75 joules per pulse) of the exposed liver were accompanied by minimal bleeding or serious effects on structures adjacent to the irradiation site. Following repair of the abdominal wall after the exposed area had been irradiated, the animals behaved normally. Survival times of these animals did not differ from controls. These observations indicated that exploratory studies of the application of laser radiation to surgical procedures were warranted.

Similar observations were made following irradiation of other abdominal and pelvic organs after surgical exposure, indicating further the potential value of laser radiation for partial resection of some structures (7, 16, 26).

Irradiation of the spleen at more than 50 joules per pulse resulted in gross effects similar to those observed on irradiation of the liver. Bleeding was somewhat more severe than in the liver, but also stopped spontaneously.

Microscopic changes noted in the liver and spleen following direct irradiation differed in degree from those found in these organs on irradiation through the intact skin (7). The lesions produced in the exposed liver or spleen by energy densities higher than 100 joules per sq. cm. consisted of superficial ulcers, which increased in depth with increasing energy density. The layer deep to the ulcer showed complete destruction of liver tissue. Residual structures identifiable as cells, were elongated, distorted and surrounded by empty spaces (7, 16, 26).

Unfocused and focused irradiation of the kidney resulted in interstitial and subcapsular hemorrhages without gross bleeding beyond the confines of the kidney. Irradiation of the full bladder did not result in immediate perforation or hemorrhage. In a few cases, shrinking of the bladder occurred followed by hydronephrosis and death (7,16). Irradiation of the exposed heart resulted in instantaneous death. Hemorrhagic lesions followed irradiation of the lungs. Following bilateral testicular and ovarian irradiation, the animals were mated. No pregnancies occurred. (16)

Litwin et al (26) studied the effects of laser radiation at 6943 Å on the surgically exposed canine liver, at energy levels of 60 and 500 joules. Both unfocused irradiation and radiation focused on the liver surface and deep to the liver surface was employed. These studies were carried out to determine the feasibility of clinical use of laser radiation for the treatment of specific pathological conditions, including metastatic tumors. Minimal bleeding occurred, in contrast to surgical incision of the liver. The lesions were initially quite friable, but increased in consistency over a period of weeks while they decreased in size. Open liver biopsies were performed immediately following irradiation and at intervals thereafter. As observed in other studies, lobular architecture remained intact for a period of time (7 days in these studies), although hepatic cell necrosis was marked. Alterations in the microscopic appearance were present over the next several months. Fibroplasia of repair

was more marked at the higher energy level. The serial liver biopsies permitted extensive consecutive histological examinations providing information on the sequences of injury, reaction and repair. Litwin et al (26, 35) observed changes in blood chemistry and liver functions which could not be attributed to the surgical insult per se. Whether direct injury to a small portion of the liver, as carried out with laser radiation, could account for changes in blood chemistry values requires further consideration. Irradiation of the exposed liver in the dog was followed by apparent normal behavior, despite the temporary changes in liver function studies during the post-irradiation period. In view of the relatively non-traumatic course of the dogs during and following irradiation possible application of laser radiation to surgical procedures warrents further exploration.

In addition to studies on the direct effects of laser irradiation, consideration was given by Litwin et al (26, 35) to the investigation of other possible problems which might arise following irradiation, (unpublished data) These include formation of adhesions, infection, possible embolization, and effects due to scattering of some of the liver tissue in the plume on irradiation. Further studies at high energy levels at 6943 Å and at 10,600 and with high power argon gas lasers are contemplated.

The findings by Litwin et al (26, 35) were subsequently substantiated by those presented by Mullings and Minton (36). The livers of several Rhesus monkeys were surgically exposed and

irradiated at high energy levels. The animals survived the procedure. Serial liver function tests and other blood chemistry determinations were carried out, although repeated open serial liver biopsies were not reported. An attempt to irradiate a tumor nodule in the liver was unsuccessful, possibly due to an anesthetic death (36).

Light and electron microscopy studies of the immediate effects of ruby laser radiation at 3 to 15 joules on the directly exposed mouse liver have been reported by Zeitch et al. (37). The beam was either focused to a spot size of 6 mm at the liver surface, or was 6 mm in diameter. Although the manner of obtaining an area of this dimension is not stated it is assumed that the beam was defocused as the ruby used was probably 3/8" diameter. The alternative of using a stop to limit the beam aperture would have almost certainly been described. Increased cytoplasmic eosinophilia and diminution of mitochondrial staining was observed on light microscopy. The electron microscopy observations included changes in intercellular spaces, alterations in cytoplasmic structures, (i.e. endoplasmic reticulum and Golgi apparatus,) in the mitochondrial structure, and nuclear membrane. Since this abstracted presentation(37) has not yet been followed by a full publication, information concerning differences observed at various energy levels, or at different regions of the lesion are not available.

This study is interesting, as it is concerned with electron microscopic studies of immediate effects of laser radiation on liver tissue. It is possible that a further, more definitive

publication, would be concerned with comparative studies, using electron microscopy, of the effects of varying the parameters of laser radiation and of other modalities, such as incoherent source irradiation, conductive heat, and electro-coagulation. Electron microscopy may provide a means for study of the similarities and differences which occur with the use of the various radiation modalities. It might provide further information concerning the site and type of energy transformation which occurs during the interaction of laser radiation and tissue and provide data regarding the processes of repair. The studies will require a consideration of scattering and attenuation of the radiation by the structures of the body wall prior to impingement on the liver, (and within the liver) as a factor in comparing the effects of irradiation of an organ through the abdominal wall and direct irradiation of the exposed organ respectively.

Parsons (30) reported a preliminary study of the effects of the ruby laser on the bladders of 4 greyhound dogs. He carried out his studies on the open dog bladder, and on a water filled bladder through a cystoscope. Definitive information that would indicate that lasers were of value in urological problems was not obtained.

Insofar as laser-fiber optic treatment of bladder tumors is concerned, however, problems associated with laser irradiation include the possibility of hemorrhage and tumor spread as well as

difficulties associated with transmission of sufficiently high energy through the fiber optics system. Damage to the ureterovesical junction is important. Even if direct fragmentation of stones had been achieved, it is difficult to consider that lasers can be used for destruction of calculi *in situ*, particularly when the possibility of destruction of the stone would be accompanied by considerable particle velocity, and a plume at high temperature. The use of continuous gas lasers should, however, be considered as an alternative to tumor cautery.

. Irradiation of the Head and Brain.

Studies of effects of pulsed unfocused and focused irradiation of the forehead in mice have been carried out by Fine and Klein (14). The hair on the forehead was clipped and the area depilated. Of 41 animals irradiated at energy levels less than 100 joules, 31 died within 24 hours of irradiation. Unfocused irradiation was followed by death within 30 seconds in 10 out of 23 animals irradiated. Neurological changes were noted in the animals which survived the period immediately following irradiation. Hemorrhages from mouth, nose and ear were observed. Although petechiae were present on the reflected aspect of the scalp, gross damage to the underlying bone was not evident, but massive hemorrhages could be seen through it. Gross intracranial hemorrhages were observed in the meningeal spaces, in the ventricles and the conducting system and within the substance of the brain. Hemorrhages at the bases of the skull, as well as within the brain substance at a

distance from the site of impact, were seen.

Microscopically, hemorrhages were seen deep to the cutaneous lesion in the muscle layer. Microscopic changes were not in the bone, but the cells of the bone marrow showed elongated and condensed nuclei. In addition to gross bleeding, microscopic hemorrhages were present in the meninges and meningeal spaces as well as within the cerebral and cerebellar cortex, within the conducting system and at the base of the brain.

The effects produced were considered as due to other than temperature elevation per se. They could perhaps be accounted for by phase transformations within an essentially closed filled cavity, resulting in pressure elevation with effects produced at some distance from the site of radiation-tissue interaction. With non-Q-switched systems, a quasi-static pressure rise can occur. With Q-switched systems a more rapid pressure rise can be produced. An approximate calculation of possible pressure amplitudes as a function of energy and distension radius has been carried out (4,16).

At energy levels exceeding 100 joules per pulse, similar results were obtained on irradiation directed at the frontal or other areas of the head. However, at energy levels in excess of 500 joules, rupture of the skin overlying the skull with folding back of the edges at the site of the rupture was observed (4, 16). In addition to the effects due to increased pressures within the cranial cavity, these observations indicate that a considerable fraction of the radiation which is absorbed between the layers of the periosteum or superficial to it may be of significance. Energy

may be entrapped between the two layers of the periosteum. If phase changes occur in this region, the pressure wave (probably quasi-static on non-Q-switched irradiation) produced may be propogated, and effect intracranial contents at some distance from the site of impact.

Similarly, energy may be entrapped between the external periosteal layer and the skin. This can occur if the periosteum is a highly reflecting and scattering medium. The radiation reflected from the periosteum may then be re-reflected from the skin layer in opposition with the periosteum due to the critical refractive angle having been exceeded, as well as for other reasons.

Should this occur, energy is consequently entrapped in a small volume. Phase changes may occur. The pressure wave produced will be dependent on the elasticity of the tissue, which is probably a function of the rate of distension. Pressure production may also be due to the volatilization of material from the surface of the periosteum on irradiation, with consequent entrapment within the region between the scalp and periosteum.

Preliminary studies indicated that of the order of 10% of the incident radiation directed at the forehead of the mouse, directly penetrated through the skull and overlying tissue, at energy levels in the 50 joule range. In control studies the exposed brain was directly irradiated at energy levels of 4 to 12 joules. Lesions produced in these animals were localised to the site of irradiation and were compatible with survival.

Studies were carried out by Zarle et al (39,40) on the effects of radiation at 20 joules per pulse at 6943 Å directed at the forehead of unshaven mice and rats. With unfocused radiation the hair and scalp of white mice was burned, but no immediate or

late effects were found in the brain. With the beam focused to a 2 mm. point on the scalp, a deep burn was produced in the skin, the cranium was intact, and acute subdural, subarachnoid, and slit-like intracerebral hemorrhages were produced in the brains of some mice, but not of rats. With the beam focused so that the focal point would be within the brain, if transmitted through scalp and cranium, acute ischemic necrosis and slit-like hemorrhages were produced in brains of rats and mice. The effects were fatal to mice within a few minutes. The rats appeared to be dazed, but were not killed. Two rats were allowed to live for eleven days and the late effects were found to show features of healing contusions of the brain.

In subsequent studies by Zarle and Hayes(41), the unshaven heads of unanesthetized white mice were exposed to unfocused and focused (into the brain) laser radiation at 30-35 joules per pulse at 6943 A. Thirty-seven animals following unfocused irradiation showed no ill effects, whereas all of the 16 animals exposed to a beam focused 2 mm. into the brain showed immediate severe neurologic symptoms which culminated in death. These results were considered predictable by a purely thermal hypothesis, without change of phase to a gaseous or vapor phase.

The brains of unanesthetized cats were exposed through the dura to graded energy fluxes from a ruby laser. A 45° cone angle was utilized in an attempt to produce a crackless deep lesion. Immediately after exposure, the animals were injected with trypan blue, and after one hour, perfused. Graded effects (intradural

and subdural hemorrhage and increased permeability as evidenced by the dye were observed. All of the lesions appeared to be in continuity. Deep lesions with sparing of superficial tissues were not observed at the energy and power density levels used in these studies.

The results obtained were in contradistinction to those found by Fine et al (16), in which lesions were produced distant from the site of impact. The differences may have been due to the fact that the foreheads were not shaved and depilated in the studies by Earl et al. (39) In subsequent studies by, Fox Hayes and Stein 42) the effects of laser radiation upon guinea pigs with and without the presence of an intact skull was compared. The animals were more severely affected when they were exposed with the intact skull, because of the added effects of pressures which were considered as being produced due to superheating. This possible mechanism for lesions distant from the site of irradiation was previously comprehensively discussed by Fine, Klein et al (7). The results obtained on direct irradiation following partial removal of the skull were also similar to those previously reported by Fine and Klein (16, 17)

It is of further interest that although under the conditions of their earlier studies Earle and Hayes (39) did not find track-less lesions within the cranium at sites distant from the irradia-ted area, Fox, Hayes and Stein (42) were now able to demonstrate lesions at distant sites within the cranium as previously reported

by Fine and Klein 4,16. Although shock waves are mentioned in the report by Fox et al (42), it is difficult to determine whether these could be produced with non-Q-switched lasers under these experimental conditions, except perhaps at the leading and trailing ends of the pressure pulse produced within the relatively closed, rigid and filled cranial cavity, as discussed by Fine, Klein et al (17). If these are produced, it would be difficult to measure them. For Q-switched systems, shock waves may be produced. Again, these would be difficult to measure.

Stellar (43) compared the effects of a ruby laser radiation on the exposed brain with those produced cryogenically with a liquid nitrogen probe. Although, as the author indicates, the mechanisms by which low temperature and laser radiation interact with tissues are entirely different, the lesions produced had certain features in common. The author found that irradiation of the intact skull resulted in distant effects in agreement with previous observations by Fine and Klein (4). Lesions in the spinal cord were also observed by Stellar (43) as reported in mice by Fine and Klein (17).

The use of pressure transducers for measurement of the pressures produced within the closed filled cranium have been studied by Fine et al (17). The presence of sonic and ultrasonic waves on laser irradiation directed at the forehead has been reported (17). Ammar et al have reported the production of pressure transients in the occipital bone of rabbits, on irradiation of the interior of the eye (44). These are further discussed in other sections.

Consequently, it can be concluded that interaction of laser radiation with an essentially closed filled cavity may result in effects distant from the site of irradiation. Phase transformation resulting in an increase in intracranial pressure, the production of sonic, ultrasonic and possibly hypersonic frequencies must be considered.

Studies on the Effects of Dyes

In view of the differential interactions of laser radiation with melanin and hemoglobin the effects of various dyes were explored (1). Applications of water black, methylene blue and Janus green to depilated skin of mice increased the size and severity of the cutaneous lesions with variable effects on deep-seated visceral lesions as compared to non-stained controls following irradiation at 28-44 joules per pulse at 6943 Å. Phenol red application did not alter the cutaneous lesions, but increased the severity of visceral lesions, while picric acid produced no changes as compared to the controls. (27).

Rosenoff et al (45) applied Evans blue to a superficial extension of (human) squamous cell carcinoma of the larynx prior to irradiation, in order to determine whether laser effects would be enhanced. The authors also injected cardio-green in order to raise the sensitivity of brain tissue to laser radiation.

Kaliper etal (4,5) investigated artificially colored (human) adenocarcinoma. Some effects were observed, but these were not as marked as the effects on naturally pigmented tumors, like a malignant melanoma. Goldman and Wilson (47) stained a (human) basal cell epithelioma with Evans blue and copper sulfate respectively, and noted increased effects. Copper sulfate was found to be irritant. Goldman et al (48) also noted blanching in a human tatto, following exposure to unfocused radiation at a power level of 10 megawatts, obtained from a Q-switched ruby laser. A similar blanching effect was observed in vitro after injecting Evans blue into an excised intradermal nevus.

The studies on the effects of dyes on the interactions of laser radiation with tissues are preliminary. Detailed investigation of mechanisms by which the various dyes differ in their effects may provide a basis for specific modification of the interaction of laser radiation with biological (or other) material. Studies at the molecular (9) and cellular level (11,16) have been initiated in this respect.

Studies at High Power Densities

Fine, Hartman, Klein and Scott (5) studied radiation effects at high peak power levels (exceeding 100 megawatts, at energy levels of 3 joules) directed at the abdomen. Deep as well as cutaneous lesions were produced. At lower peak power levels (approximately

10 kilowatts at 7 to 10 joules) considerably less or no damage to the skin was observed. Focused radiation at the lower peak powers resulted at times in injuries to the underlying subcutaneous and deeper structures. However, the deep lesions produced at high peak powers were considerably more marked than at the lower peak power levels. These studies indicated that the power and power density of the radiation, as well as energy and energy density, is of importance in the interaction of electromagnetic radiation with biological systems. The threshold for a particular biological effect at which power level becomes a parameter of consequence at a specific energy level requires further study.

Ulmten et al (49) compared the effects of ruby laser radiation on rat tissues and mouse melanoma with pulse widths of several hundred microseconds and 100 nanoseconds, respectively. Goldman (40) produced transient, edematous blanched areas in a dark tattoomith a Q switched ruby laser radiation at power levels of 10 MW. These presentations are discussed in other sections of this report (Experimental Tumors, Clinical Studies).

Miscellaneous Studies

Studies at pulse repetition frequencies exceeding one pulse per second were carried out by Main, Fine, and co-workers (7, 11, 16). The effects do not appear to be quantitatively similar when the same energy (of the order of 300-500 joules) is delivered at pulse repetition frequencies of 1 pulse per second (several joules per pulse) as compared to delivery of this energy as a single pulse of

millisecond duration. One of the reasons for this may be that interaction of a pulse with tissue can alter the tissue in such a manner that the absorption of radiation during the following pulse differs from that of the previous one.

Klein, Fine and co-workers (7, 11) investigated the effects of single pulses at 6943 Å and at energy levels ranging from approximately 30 millijoules to 100 joules on hemostasis and skin flaps in normal mice. Irradiation of skin flaps in which adequate circulation was maintained, resulted in the production of intravascular thrombosis. The degree of injury to the blood vessel wall varied from non - detectable to frank interstitial hemorrhage. Factors affecting these results included direction of the beam, energy level, spot size and size of the artery or vein. With the laser units focussed through a microscope, vasoconstriction, thrombosis and interstitial hemorrhage was observed.

Hemorrhage produced in the tail was studied at energy levels in the 50 joule range (7). Focussed radiation produced hemostasis in heparinized animals, while unfocussed radiation at these energy levels did not stop the bleeding.

Soroky, Cohn and Thomas (9) explored the possibility of utilizing radiation at 10,600 Å for anastomosing small blood vessels. The vessels were approximated side to side, and the common wall was

exposed to laser radiation through the open end of the donor vessel. A fistula was produced and the open end of the donor vessel was closed. These early explorations will require considerable additional studies to assess the feasibility of such an approach to vascular surgery.

Peres (37) irradiated the appendix and cyphoi area of rats at energy levels of 0.3 joules per pulse at 6943 Å. The areas were exposed to multiple impacts at 15 second inservals for 5, 30 and 60 minutes. The following determinations were carried out: hematocrit, serum protein, BUN blood sugar, liver and muscle glycogen, gastric absorption of isotonic solution of glucose, BSG, respiration and weight of the adrenals. No significant changes were found as compared to normal values. The authors suggested that these tests could be repeated at higher energy levels.

Immunological effects of laser radiation on tumors and normal muscle tissue were studied by Lord and Kinsey(31) in inbred rats. Klein, Fine et al (30) previously reported regression of non-irradiated Rudding-Passey melanomas after one of 2 or more tumor nodules in the same mouse had been exposed to laser radiation (6943 Å, 300 joules per pulse, pulse width of msec.) These effects were probably due to latent immunological incompatabilities between the tumor and the host. The sudden removal of one tumor may have made it possible for an otherwise

inadequate antigenic response to inhibit the remaining tumors. Similar phenomena have been reported following surgical removal or x-irradiation of a tumor in animals with multiple (transplated) neoplasms. There is no evidence that laser radiation alters a specific tumor protein to induce a selective antigenic response.

Comparative Studies.

In view of the relative mildness of superficial lesions in the presence of severe deep-seated lesions following laser irradiation of the intact animal and in view of the discontinuity of the lesions along the direction in which injury had taken place, comparative studies on the direct application of conductive heat were carried out (16,25). These studies were initiated since data on thermal trauma in mice were not available. Lesions were induced by monopolar or bipolar electrocoagulation current. The cautery needle was applied manually through the skin to the underlying tissue for the shortest time possible. Lesions were also induced by a steel rod which had been kept in boiling water prior to application or by pipetting of boiling water onto the surface of the skin. Lesions were further induced by flash tube irradiation and exposure to a mercury arc. The injury produced by electrocautery was relatively uniform throughout the affected tissues as represented by a continuous coagulum along the path of the

cautery needle. The distortion of the epidermis was similar to that found in severe laser injury. The dermis showed homogeneous coagulation. Swelling of the adipose cells in the subcutaneous layer was marked. The muscle had lost its striations and vacuoles were found within the muscle fibers. The lumina of the blood vessels were contracted and empty and thus hardly recognizable.

The changes following injuries with boiling water or hot steel showed separation of the epidermis from the dermis without empty spaces or spongiosus within the epidermis. The superficial lesions were relatively mild and the muscle layer appeared to be unchanged.

The animals which had been exposed to flash tube irradiation at energy levels up to 5,000 joules per pulse survived these exposures without apparent internal injuries. Regrowth of the hair was accompanied by diffuse loss of pigmentation. This was in contrast to animals which had been exposed to laser radiation in which, at considerably lower energy levels, internal injuries were produced and depigmentation was sharply localized.

Exposure of the mercury arc at 100 watts for 60 seconds (53) produced a continuous zone of almost total coagulation which extended through the muscle. The distortion of the epidermis and epidermal appendages did not seem to be as marked as at comparable levels of laser energy. Ruptured blood vessels in the muscle layer which were produced by laser radiation, were not observed following mercury arc irradiation, although the changes in the muscle fibers were comparable in severity.

The various comparative studies failed to induce the differential effects on certain types of tissues, which were produced by laser radiation.

Conclusions

Further studies on animals are required to investigate the mechanisms of the interaction of laser radiation and to delineate hazards. Studies on small animals present data on "scaled down" model systems, which were suitable for exploration of the effects of laser devices with relatively low energy and power output available at that time. Direct extrapolation of these observations to effects of radiation on larger animals or man at higher energy and power levels is difficult. Since technological advances have made high energy and power levels available, studies on larger animals have been initiated. Comparative studies with Q switched and normal mode laser systems should be intensively pursued. In vivo effects and hazards associated with gas laser systems, some of which are currently operating at power levels exceeding 20 watts, require exploration. These studies will be of significance in regard to the effects of laser radiation on man.

Table I: Laser Irradiation of the Skin

Group No.	Irradiation Site	Total Number of Animals	Color Black - White		Energy Levels (joules)	Irradiation Type Focused - Unfocused		Remarks
1	Abdomen	80	40	40	70-100	40	40	Equal numbers sacrificed immediately and at 1, 3, and 5 days; 8 died prior to sacrifice.*
2	Abdomen	28	14	14	70-100	10	18	Kept for long term evaluation (since December 1963).
3	Abdomen	11	6	5	350-860	2	9	5 died immediately; 5 died within 3 days; 1 alive 15 months later.**
4	Abdomen	46	20	26	20-70	4	42	Sacrificed at intervals from immediately to 26 days.
5	Abdomen	50	20	30	7-20	15	35	12 sacrificed from immediately to 8 days; rest kept for long term evaluation.
6	Chest wall	9	3	6	70-100		9	2 died within 2 days; 7 are alive 16 months later.***
7	Chest wall	7	3	4	350		7	4 died within 2 days; 3 alive 15 months later.***
8	Forehead	48	24	24	70-100	20	28	39 died within 1 day; 3 died within 5 days; 6 alive 16 months later (2 have neurological symptoms).****
9	Forehead	3	1	2	860	1	2	All died immediately.*****
TOTAL		282				92	190	

* Died of peritonitis from intestinal perforation[7]
** Immediate death from intraperitoneal hemorrhage; delayed death from peritonitis[7]
*** Died of lung hemorrhage or myocardial necrosis[7]
**** Died of subarachnoid, intraventricular and intracerebral hemorrhages[22]

REFERENCES

1. Fine, S., Klein, E., Scott, R.E., Roy, A., Some effects of laser radiation on the skin of the Syrian hamster, Life Sciences, 1:30-35, 1963.

2. Fine, S., Klein, E., Scott, R.E., Seed, R., Laser radiation in the Syrian hamster, Skin, 2:43-48, 1963.

3. Fine, S., Klein, E., Farber, S., Scott, R.E., Roy, A., Seed, R., In vivo effects of laser radiation on the skin of the Syrian hamster, J. Invest. Dermat. 40:123-124, 1963.

4. Fine, S., and Klein, E., Effects of pulsed laser irradiation of the forehead in mice, Life Sciences, 3:199-207, 1964.

5. Fine, S., Maiman, T.H., Klein, E. and Scott, R.E., Biological effects of high peak power radiation, Life Sciences, 3:209-222, 1964.

6. Fine, S., Klein, E., Hardway, G., Scott, R.E., King, W., Aaronson, C., Use of closed circuit television in laser investigations, J. Invest. Dermat. 4:289-291, 1964.

7. Fine, S., Klein, E., Nowak, W., Scott, R.E., Laor, Y., Simpson, L., Crissey, J., Donoghue, J. and Derr, V.E., Interaction of laser radiation with biologic systems. I. Studies on interaction with tissues, Fed. Proc. 24 (1, pt.3), supp 14:s35-45, 1965.

8. Klein, E., Fine, S., Laor, Y., Simpson, L., Ambrus, J., Richter, W., Smith, G.K., Aaronson, C., Interaction of laser radiation with biologic systems. II. Experimental tumors, Fed. Proc. 24 (1 pt. 3), suppl 14:s143-149, 1965.

9. Klein, E., Fine, S., Ambrus, J., Cohen, E., Meter, E., Ambrus, C., Bardos, T., Lyman, R.: Interaction of laser radiation with biologic systems in vitro, Fed. Proc. 24(1, pt3), suppl 14:s104-110, 1965.

10. Derr, V.E., Klein, E., Fine, S.: Free radical occurrence in some laser irradiated biologic materials, Fed. Proc. suppl 14:s99-103, 1965. (24, (1,pt. 3).

11. Fine, S., Klein, E. and Scott, R.E.: Laser Irradiation of biological systems, IEEE Spectrum, 4:81-95, 1964.

12. Nowak, W.B., Fine, S., Klein, E., Bergenrother, K., Hansen, W.P, On the use of thermocouples for temperature measurement during laser irradiation, Life Sciences, 3:1475-1481, 1964.

13. Fine, S., Nowak, W., Hansen, W., Hergenrother, K., Scott, R.E., Donoghue, J. and Klein, E.: Measurements and hazards on interaction of laser radiation and biological systems, NEREM Record, 6:158-9, 1964.

14. Derr, V.E., Klein, E. and Fine, S.: Presence of free radicals in laser irradiated biological specimens by electron spin resonance. App. Opt. 3:786-787, 1964.

15. Edlow, J., Fine, S., Vawter, G.F., Jockin, H. and Klein, E.: Laser irradiation: effect on rat embryo and fetus in utero, Life Sciences, 4:651-623, 1965.

16. Fine, S. and Klein, E: Biological effects of laser radiation, Advan. Biol. Med. Phys. (in press).

17. Fine, S., Klein, E., Fine, B.S., Litwin, M., Nowak, W., Hansen, W.P., Caron, J. and Forman, J.: Mechanisms and control of laser hazards and management of accidents. Monograph of the 2nd National Conference on Laser Techology (in press).

18. Fine, S., Klein, E., Ambrus, J.L., Cohen, E., Ambrus, C., Derr, V.E., Nowak, W.: Interaction of relatively coherent laser radiation and biological systems. Fed.Proc. 23:442, 1964.

19. Klein, E., Fine, S., Scott, R.E., Farber, S.: Observations of laser irradiation on experimental tumors, presented at the American Association for Cancer Research, April 1964, Chicago, Illinois.

20. Klein, E., Fine, S., Effects of laser radiation on animal tissues, presented at Conf. Laser, N.Y. Acad. Sci, New York, May 4-5, 1964.

21. Fine, S., Klein, E., Laor, Y., Modification of effects of laser radiation by light absorbing chemicals, presented at Boston Laser Conf. 3rd, Boston, Massachusetts, August 5-7, 1964.

22. Klein, E. and Fine, S., Laser irradiation in biology and medicine, presented at Ann. Meeting Radiol. Soc. N. Am., Dec. 4, 1964.

23. Klein, E. and Fine, S., Laser radiation in Dermatology, presented at Ann. Meeting Am. Acad. Dermatol., Chicago, Ill., Dec. 7, 1964.

24. Klein, E., Fine, S.:The biological aspects of laser radiation. Absts. Meeting Am. Chem. Soc., 149th, Detroit, Michigan, April 9, 1965.

25. Laor, Y., Simpson, L.C., Klein, E. and Fine, S., The pathology of laser irradiation of the skin and body wall of the mouse, Am. J. Pathol. (in press).

26. Litwin, M.S., Fine, S., McCombs, H.L., Klein, E., Effects of laser radiation on the surgically exposed canine liver, Fed. Proc., 24: 556, 1965.

27. Klein, E., Fine, S., Loor, Y., Litwin, M.S., Donoghue, J. and Simpson, L., Laser irradiation of the skin, J. Invest. Dermatol., 43:565-570, 1964.

28. Edlow, J., Fine, S., Vawter, G.F., Laser irradiation: effect on liver of neonatal rat, Fed. Proc. 24, 556, 1965.

29. Goldman, L., Balney, D., Kindel, D., Richfield, D., Franke, E., Pathology of the effect of the laser beam on the skin, Nature, 197:912-914, 1963.

30. Helwig, E., Jones, W., Hayes, J., Zeitler, E., Anatomic and histochemical changes in skin after laser irradiation. Fed. Proc. 24 (1, pt 3) suppl. 14:583-591, 1965.

31. Moritz, A.R., The pathology and pathogenesis of cutaneous burns an experimental study, Amer. Journ. of Pathology, 23:915-941, 1947.

32. Sheline, G., Alpen, E., Kuhl, P., Ahokas, A., Effects of high intensity radiant energy on skin I. Type of injury and its relation to energy delivery rate, A.M.A. Arch. Pathol. 55:265-70, 1953.

33. Alpen, E., Sheline, G., Kuhl, P., Ahokas, A., Effects of high intensity radiant energy on skin II. Quantitative Dependence of tissue injury on duration of exposure, A.M.A. Arch. Pathol., 55:280-285, 1953.

34. Bell, Eugene, Action of ultrasound on adult and embryonic organ systems, Am. J. Phys. Med. 37:184-191, 1958.

35. Litwin, M.S. (in preparation).

36. Mullins, F.X. and Minton, J.P., The effect of multiple high energy laser pulses on the primate liver, Proc. Biomedical Laser Conf. Laser Med. Res. Found, June, 1965.

37. Faith, G.C., Hayes, J.R. and Stein, M.H.: Immediate effects of laser irradiation upon mouse liver: light and electron microscopic observations, Fed. Proc. 24:238, 1965.

38. Parsons, R.L., Preliminary study of urologic applications of the laser: effect of ruby laser radiation on the open dog bladder, Proc. Biomedical Laser Conf., Laser Med. Res. Found., June 1965.

39. Earle, K.M., Sterling, C., Rosenmann, U., Ross, M.A., Hayes, J.R., and Zeitler, E.E., Central nervous system effects of laser radiation, Fed. Proc. 24 (1 pt 3) suppl. 14, 5-129, 1965.

40. Earle, K.M. and Hayes, J., Abstr. 3rd Boston Laser Conf., 1964.

41. Earle, K., Carpinter, S., Roessmann, U., Ross, M., Hayes, J., Zeitler, E., Central Nervous system effects of laser radiation, J. Neuropathology and Exploratory Neurol. 24:164-165, 1965.

42. Fox, J.L., Hayes, J.R. and Stein, M.N., The effects of laser radiation onintracranial structures, Proc. Biomedical Laser Conf.,Laser Med. Res. Found., June 1965.

43. Stellar, S., Effects of laser energy on brain and nerve tissues, Proc. Biomecial Laser Conf., Laser Med. Res. Found., June 1965.

44. Amar, L, Bruma, M., Desvignes, P., Leblanc, M., Perdriel, G., Velghe, M., Detection of elastic waves (ultrasonic) in the occipital bone induced by laser pulses in the eye of a rabbit, C.R. Aca. Sc. Paris 259 (2):3563-3565, 1964.

45. Rosenoff, H., Hellstrom, R., Brown, J., Carroll, F., Effect of laser on carcinoma in man, J. Am. Med. Assoc. 192(2):167, 1965.

46. Helsper, J., Sharp, G., Williams, H., Fister, H., The biological effect of laser energy on human melanoma, Cancer 17:1299, 1964.

47. Goldman, L., Wilson, R., Treatment of basal cell epithelioma by laser radiation, J. Am. Med. Assoc., 189:773, 1964.

48. Goldman, L, Igelman, J., Richfield, D., Impact of the laser on nevi and melanomas, Arch. Dermatol. 90:71, 1964.

49. Minton, J.P. and Ketchum, A.S.: A comparison of the effect of microsecond and nanasecond ruby laser radiation on rat tissues and mouse melanoma, J. Surg. Res. 4:281-285, 1964.

50. Strully, K.J., Yahr, W.Z. and Burnitt, E.S., Experimental cardiovascular laser surgery: a method for small blood vessel anastomosis utilizing laser and monomer, Proc. Biomedical Laser Conf., Laser Med. Res. Found., June 1965.

51. Peres, C., Croumoulon, G., Dumas, J., and Piolat, J.: Les effets biologiques du rayonnement laser. I. exploration de quelques fonctions chez l'animal, C.R. Soc. Le Biol. 158:2111-21113, 1964.

52. Bard, D.S. and Minton, J.P., The immunological effects of the laser and cautery on normal and neoplastic tissues in inbred rats: preliminary report, Proc. Biomedical Laser Conf., Laser Med. Res. Found., June 1965.

53. Hust, F., Lyman, R., Klein, E., Fine, S. and Laor, Y. (in preparation).

Studies on the effects of laser radiation of malignant tumors transplanted to the cheek pouch of the hamster were carried out as part of initial explatory experiments by Fine, Klein et al (1,2,3) of the interactions of coherent electromagnetic radiation with biologic systems. The earliest studies (1) indicated pronounced destructive effects on tumors, while irradiation of the normal cheek pouch, under otherwise the same experimental conditions, produced relatively minor changes. The differences in effects on tumors and normal tissues, respectively, were related to differences in architecture, the nature of the blood supply, and the presence of pigments acting as absorbents of the radiation. These studies provided no evidence that the marked changes in tumors were due to characteristics of malignant cells per se, as compared to normal cells.

These exploratory studies (1,2) suggested, however, that some properties of tumors were more suitable than normal structures for the investigation of the interaction of laser radiation with tissues. The relatively large cell population of tumors is more readily accessible in small laboratory animals than normal cell populations of comparable size. Inhibition of growth is an easily measurable parameter of biologic activity. Even a few tumor cells surviving exposure to radiation, furthermore, would manifest themselves by growing out to appreciable dimensions. Some components of tumors, such as pigment in the melanomas, may behave as energy absorption and transfer agents thus induce differential interaction with the radiation (4). Subsequent studies (5,6,7,8) indicated that extensive damage to neoplastic tissues could be produced by laser radiation in several transplanted mouse tumors as had been previously observed in tumors in the hamster (1).

Since these initial studies (1,2,3) the effects of various types of laser radiation on experimental tumors and on cancer in patients has drawn increasing interest. As in any new area of investigation, the effects of laser radiation on tumors require intensive investigation for a clearer understanding of the physical and biological factors underlying them. The possibility of meaningful clinical application of lasers to the management of cancer in humans in particular, will depend on defining indications and contra-indications. Extensive studies on experimental tumors in animals will furnish some of the information for guiding a justifiable approach to clinical investigation.

In most of the studies by Klein, Fine et al (8) pulsed laser radiation at 6943 Å and 10600 Å was used. Energy levels ranged from 10 to 900 joules per pulse and up to 2000 joules in total energy. Most studies were carrried out at power levels of the order of 10-100 kw. Successive exposures were carried out at intervals ranging from 1 sec to several days. Radiation was focused by simple lens systems or was unfocused. Spot size ranged from 3 to 14 mm in diameter. Exploratory studies were carried out at 6943 Å with Q-switched radiation at energy levels of 4 joules per pulse and peak power levels ranging from 50 to 500 MW. The spot sizes were approximately 10 mm in diameter, whether the Q-switched radiation was unfocused or defocused. Studies were also carried out with an argon gas laser (5100 Å) and a helium neon gas laser operating at 6328 Å and a nitrogen gas laser operating at 3371 Å.

Studies by Klein, Fine, et al (8) were carried out on the effects of laser radiation in more than 1,000 tumor-bearing animals in addition to normal controls and tumor-bearing controls which were not irradiated.

Although Fine, Klein, et al (1) had observed significant effects of laser radiation on tumors in the hamster cheek pouch, most of their subsequent work was carried out on tumor-bearing mice. The reasons for emphasizing studies in mice were the availability of a large number of inbred strains and established tumor lines in that species. Defined biological characteristics and considerable background information from the oncological literature could therefore be utilized. The tumors under study were Harding Passey and Cloudman S_{91} melanomas, Ridgway osteogenic sarcoma, and Lewis bladder carcinoma. Transplants were carried out in the following strains of mice: Swiss (Harding Passey, Ridgway); DBA/1 (Cloudman S_{91}); and C_{57}/6 (Lewis bladder carcinoma). Irradiation was carried out at various intervals after transplantation. In several groups, multiple transplants were symmetrically located to provide control systems. Irradiation of tumors was carried out through the intact skin, except for some studies in which the skin and subcutaneous tissues were incised and retracted to expose neoplastic tissue directly to the radiation.

In an early study (5), four groups consisting of 50 Harding Passey melanoma-bearing mice were irradiated. The diameter of the cross sections of the tumor nodules varied from 7 to 25 mm. Group 1 consisted of 18 animals exposed to a single exposure ranging from 350 to 1,200 joules. After two weeks, regression of the tumor was noted in 13 animals. Regressions were complete within six weeks, Two of the animals, which showed no regression, had considerably larger tumors prior to irradiation than the average (25mm. in diameter); three animals died within 24 hours, presumably due to the trauma of the irradiation.

Group 2 consisted of seven animals exposed to high pulse repetition frequencies at energy levels ranging from 1,000 to 2,000 joules. Three of

these animals died within 24 hours after irradiation, and all seven were dead within six days, presumably due to the trauma of irradiation. Necrosis was more severe in the irradiated parts of the tumors in this group than in groups 1 or 3. At autopsy, five of the mice irradiated at high pulse repetition frequencies had extensive hemorrhages and other injuries in viscira located deep to the irradiated tumors.

Group 3, consisting of 20 animals, received multiple exposures at intervals of 3 minutes; the total energy varied from 10 to 620 joules. Regressions were observed in three animals which had received a total of more than 300 joules. The cross sections of the tumors were less than 10 mm. in diameter. The estimated depth, to which the tumors extended, ranged from 3 to 7 mm. The course of irradiated tumor-bearing animals which did not show regressions was essentially similar to the course of non-irradiated, tumor-bearing controls.

Group 4 consisted of five animals that received multiple exposures at energy levels of 160 to 220 joules. Four exposures were carried out on each animal at intervals of 24 hours. Regression occurred in two mice in this group; the remaining three animals died due to extensive tumor growth.

A total of 16 animals had been implanted with two or more tumors; one tumor in each animal was irradiated. Six of these animals showed regressions in both the irradiated and non-irradiated tumor. In the remaining ten animals, both tumors continued to grow and killed the host. Regression of multiple implants is known to occur following surgical removal or X-ray exposure of one of the tumors, and is therefore not unique for laser irradiation.

In animals with one or more tumors in which no regression occurred, irradiation was followed immediately by a lesion which involved the skin and underlying tumor tissue. These lesions were 3-7 mm. in diameter and depth, with a narrower extension for another 5 mm. or more, depending on energy and direction of the radiation. For the next 24-48 hours, the size of the necrotic area of the tumor increased, accompanied by progressive breakdown of the overlying skin and subcutaneous tissues. The necrotic area then became covered by an eschar, which remained stationary or gradually increased in size until death. The residual, viable part of the tumor, not involved by the primary effects or by secondary necrosis, continued to grow at the periphery of the laser-induced necrotic area.

Microscopically the central area of the laser-induced necrosis differed from the necrosis in control non-irradiated tumors by its relatively smooth outline due to the absence of the usual perivascular extensions of surviving cells; superficially, changes similar to those in epidermis after laser irradiation sometimes occurred. Distorted cells surrounding small vesicular spaces were seen and these retained nuclear staining longer than the deeper necrotic areas, which appeared to be due to spontaneous necrosis.

The initial local reaction to the radiation appeared to be similar whether or not it was followed by regression. However, after 2-3 weeks, regression of the tumor was suggested by lack of extension of necrosis and decrease in size of palpable tumor. Healing with scar formation followed. By the time healing was apparently complete, the tumors were no longer palpable. Regressions persisted during observation periods of more than 24 months. The non-irradiated controls and the irradiated tumor-bearing animals, which had not shown regression, died during the expected period of time (i.e. 3 months following implantation of Harding-Passey melanoma).

Biopsies were taken from irradiated sites in 16 animals with regressed Harding-Passey melanomas, 1-6 months after irradiation. These animals remained alive except for one animal, which died under anesthesia. Biopsies of the 16 animals showed microscopic evidence of a previous lesion, but no viable tumor cells. In the sections of six animals, groups of melanin-containing histiocytes were present in the subcutaneous tissue. In seven animals, mild, chronic inflammatory changes were found while in two animals the only evidence of a pre-existing lesion were areas of dense collagen in the dermis and subcutaneous tissue, and absence of the normal appendages of the epidermis. In three animals, hyperplastic changes were present in the epithelial cells of the skin appendages.

A group of 35 animals with Cloudman S_{91} melanomas received multiple exposures to unfocused radiation of 10-75 joules per pulse. Exposures were at intervals of 24-48 hours, and total energy levels ranged from 30 to 250 joules. In one of these animals, the irradiated tumor nodule appeared to decrease in size; however, metastatic lesions developed in areas adjacent to the irradiation site as well as at more distant locations. The course of the other 34 animals did not substantially differ from that of the non-irradiated controls. These observations differ from those of Minton et al. (9) and from the results of later studies by Klein, Fine et al. (10). The lack of regression in the earlier studies on S_{91} (Cloudman) melanoma is due to the relatively low energy and power levels of the radiation and the large sizes of the tumors. The advanced stages, at which S_{91} tumors were irradiated in the early studies by Klein, Fine, et al. (4) furthermore indicated that metastatic extension is likely to have taken place prior to irradiation. Therefore, even complete resolution of the primary implant following laser irradiation would not have effectively altered the course of the disease.

A group of 22 animals with Ridgway osteogenic sarcoma were irradiated four weeks after implantation. Eleven animals were exposed to a single irradiation at 100 joules aimed at the center of the tumor. Regression was observed in one animal. The other 10 animals died due to the continued growth of the tumor. Seven animals were exposed to single exposures at 350 to 900 joules. Regressions were noted in four animals. Four animals received 10 exposures at 100 joules per pulse at five minute intervals. Each exposure was directed to the same area of the tumor. No regressions were noted in this group.

The gross effects of laser irradiation of Ridgway osteogenic sarcoma were similar to those observed in progression and regression, respectively, of irradiated Harding-Passey melanoma. Biopsies taken from five sites, at which regression of Ridgway sarcoma had taken place, showed scar formation, chronic inflammation, and histiocytes containing hemosiderin.

Lewis bladder carcinoma was studied in 23 animals. Irradiation was carried out four weeks after transplantation. Three animals were exposed to single pulses varying from 900 to 1,200 joules. No regressions were observed in these animals. Thirteen irradiated animals received multiple exposures with total energy levels ranging from 40 to 200 joules. Regressions occurred in two animals. The other irradiated tumors in this group continued to grow as in non-irradiated controls until death of the animals. Three animals were exposed to high pulse repetition frequencies at low energy levels per pulse (total energy 300 to 1,500 joules). The animals died within 48 hours, probably due to trauma of the irradiation. Autopsy revealed extensive hemorrhage and injuries to underlying viscera as had been seen in the normal and Harding-Passey-bearing mice irradiated at high pulse repetition frequencies.

In these studies, except where death was immediate, a primary lesion was seen immediately after irradiation, followed by more extensive necrosis

within 24 hours. The survival time of irradiated animals in which no regression occurred was shorter than, or fell into the same range as the survival time of non-irradiated controls.

Although information regarding the irradiation energy is given, wavelength, energy density and size of tumor for each group irradiated is not well defined. Some consideration is, however, given to these factors in the author's discussion. Since the method of energy measurement is not given, it is not possible to determine the accuracy of the energy values stated. Although beam splitters were used to measure the energy, particularly at high energy levels, inaccuracies in measurement of high energy levels may have been considerable. Since high energy irradiation were carried out at other laboratories than those of the investigators, often with prototype equipment, the exact values given are questionable. The data are, however, stated by the authors as being preliminary.

These observations (4) were confirmed and extended in subsequent studies by Klein, Fine, and their associates (8,10) on more than 500 tumor-bearing animals. It was apparent from these studies that energy and power density, size of irradiation field, size of tumor, capacity for local or metastatic tumor growth, stage of tumor development, presence of pigment, nature of blood supply and anatomical location of tumor were interrelated factors in regard to the effects of laser radiation on normal tissues or on tumors.

In recent exploratory studies by Klein, Fine, Paananen, et al. (10), the effects of continuous radiation in the blue-green region of the spectrum obtained from an argon gas laser were investigated in normal mice and tumor-bearing animals (Harding-Passey melanoma, S_{91} Cloudman melanoma). Exposure to a power output of 5W for 5-20 seconds resulted in regression in 5 out of

10 Harding-Passey melanoma-bearing mice. No regressions were observed in 10 animals with S_{91} Cloudman melanoma under these conditions of irradiation.

Following the initial report by Fine et al. (1,11), McGuff et al.(12) reported studies on experimental tumors transplanted to the cheek pouch of the hamster. These tumors included carcinoma of the thyroid and breast, and melanoma of human origin. Energy levels obtained from a ruby laser with an output of up to 900 joules per pulse were employed. Complete regression of the irradiated tumors were reported to follow exposure to single pulses. Control studies are not presented in this report (12). These would have been particularly indicated, since heterologous tumors (human) in the hamster have a high spontaneous regression rate due to immunogical incompatabilities. A few human tumor lines have become established in the hamster, but no statement was made that the tumors used by McGuff et al.(12) were obtained from established lines. In a subsequent report, McGuff et al.(13) present observations on tumor-bearing animals including non-irradiated controls. In these studies, the irradiated tumors underwent regressions, while the non-irradiated control tumors continued to grow. Transplanted tumors of hamster origin initially induced with chemical carcinogens, were included, presumably to avoid immunological incompatibilities. Since Syrian hamsters (unlike mice) are not available as inbred lines, transplantation of tumors of hamster origin reduces, but does not exclude the role of immunological factors in tumor regression. McGuff et al.(14) subsequently compared the effects of laser irradiation with those of x-rays (1000R) alone and of x-rays in combination with laser radiation. The laser irradiated tumors were reported to have shown earlier regressions than tumors exposed to a combination of x-radiation and laser radiation. Tumors exposed to x-rays alone did not regress, but

continued to grow at a slower rate than the non-irradiated control tumors, as expected, since a tumoricidal dose was not used. It is of interest in this connection that Rounds et al.(15) concluded that gamma radiation and laser radiation acted synergistically in damaging cells in tissue culture.

In the studies on tumors in the hamster cheek pouch by McGuff et al (12) no reference was made to the characteristics of the blood supply. The blood supply to the cheek pouch, and therefore to the tumors implanted in it, arises from the blood vessels in the base of the membrane. The major blood vessels are exposed and as shown in previous studies on the normal cheek pouch by Fine et al. (1) are subject to serious damage by laser irradiation. It would therefore be important to determine whether the effects of laser radiation on the tumors implanted in the cheek pouch is due to direct action on the tumor or to damage of the blood supply which would secondarily result in tumor death, or to both. There is no evidence of control studies in the reports by McGuff dealing with the normal (i.e. free of tumor) hamster cheek pouch. The resultspresented by McGuff et al. (12,13,14) do not relate the parameters of the radiation, such as energy per pulse, total energy, spot size, etc., with size of the irradiated tumor. The sizes of the tumors irradiated, furthermore are not indicated. It is therefore difficult to assess whether only part of the tumor was irradiated, and the remainder regressed although it had not been directly irradiated, or whether regression took place as a result of the irradiation covering the entire area involved by tumor. No indications are given furthermore of the depth to which the tumor had penetrated. It should also be noted that a majority of the tumors implanted in the cheek pouch, particularly those of human origin, do not metastasize but grow as expanding lesions. These studies therefore do not present indications of the effects of laser radiation in regard to advancing

or inhibiting the rate of metastatic invo ment. It is possible that laser irradiation may result in the separatior viable tumor cells, which may then lead to dissemination of the tumor. Si cGuff et al. (16,17,18) carried out concurrent studies on malignant tum in man, it would have been important to obtain this type of information animal stud .

Statistical analyses of this data are neither presented nor mentioned. Since the data obtained by McGuff et al. in several of their studies (12,18) are close to the all-or-none type, statistical analysis would have been particularly meaningful as well as impressive. Presumably the lack of statistical evaluation may be related to the paucity of control studies presented.

Further data presented by McGuff et al. (19,20) indicate that complete regression of the tumor is identified as a lack of residual tumor cells on excisional biopsy. After excisional biopsy has been performed, it is obviously impossible to determine recurrence rates of non-metastasizing tumors (as were the majority of those studied); since recurrence would have to be local. It is well known that particularly tumors of human origin can remain dormant in the hamster for up to one year or longer. Thus the lack of apparent tumor cells does not exclude the possibility of their continued presence and eventual reactivation into a growing tumor. Since, furthermore, McGuff's data do not indicate that serial sections or serial blocks were examined, it is difficult to exculde the possibility of residual tumor cells even at the time at which the biopsy was obtained, regardless of whether eventual recurrence would have taken place or not.

The paper by McGuff et al. in the Annals of Surgery (18) includes the are material as presented in the Canadian Medical Association Journal by the rs (13), although in the former paper irradiation of carcinoma of the in the hamster cheek pouch was not mentioned. Since the methods and

the discussion in the two papers are virtually the same, as well as the data, the critique of one of these papers (19) which is presented in another section of this report will not be repeated here. In brief, however, the statement is made that 20 different types of tumors were studied in 700 animals in one or two anatomical locations. Assuming uniform distribution, an average of 35 tumors of each type (and of unnoted size) were treated with from "60 to 180 joules per burst" with one or more exposures. Thus at least three variables would have been studied in 20 animals assuming 15 controls were used. This would leave approximately six animals per parameter studied. It would be difficult to see how six animals would be sufficient to obtain significant data on radiation parameter and tumor characteristics. Should more than one parameter have been varied (no statement was made as to which of these might have been kept constant), the number of combinations of variables would reduce the number of data to observations on one or two animals for each experimental condition.

Of 20 different types of tumors in 700 animals, two groups of animals are presented in specific numbers. In the section dealing with "adenocarcinoma of human origin" the nature of the adenocarcinoma is not stated. The range of energy is stated as "required" without indication how this level of energy was determined nor for what effect it was required. Since the number of animals available for this study was 16, it would clearly have been impossible to determine sequentially the response to increasing amounts of energy over that wide a range. The statement is furthermore made that the average amount of energy "required" was 250 joules per tumor. It is difficult to reconcile these statements with the number of exposures specifically stated as having been carried out.

In the section on malignant melanoma 3 of 18 irradiated animals died by the seventh post irradiation day and two more animals died by the third

post irradiation week. The causes of death are not indicated. Again the physical parameter (energy per pulse, number of exposures, cross-section of the spot size, etc.) and the biological characteristics of the tumor (such as cross-section, depth, time following implantation, etc.) are not stated. Of 17 animals referred to as controls, five animals were kept for "long time study" (8½ months), but no statement is made as to the fate of the other 12 "control" tumors.

Preliminary analysis of the data is stated to indicate that temperatures probably do not exceed 46°C one second after the laser "burst". No indication is given how these temperature measurements were carried out.

Several of the observations mentioned in the papers by McGuff et al had previously been reported by a number of other authors, but are not referenced in regard to their source. Other observations made by different groups, which bear directly on the work by McGuff et al, are not referred to by the authors.

Ketcham, Minton, and their collaborators (21) reported studies on several experimental tumor systems, particularly Cloudman S_{91} melanoma and T-241 sarcoma. Their studies explored the possibilities of correlating probability of inducing regression with the absorption characteristics of the tumor tissue and the energy density of the radiation. An attempt was made to predict the energy required to destroy tumor. Minton et al (22) have attempted to relate the energy required to destroy a tumor to tumor diameter. The relation between tumor diameter and tumor volume is not discussed. Since the extent to which the tumor is below the surface has not been related to tumor diameter, the relation of tumor diameter and tumor volume has consequently not been established. From the data, therefore, it is not possible to determine the energy required to destroy a tumor of a specific volume (size).

Although Minton et al (23) emphasize the significance of the consistency of the tumor in regard to the effects of laser radiation, the model developed for predicting tumor destruction does not appear to take into account the location of the tumor, particularly in regard to whether the underlying structures are hard or soft. Since Minton et al (23) indicated that other than thermal effects per se, such as pressure waves, are of significance for tumor destruction,, in agreement with studies by others (4,24,25), the site of tumor location and the consistency of the surrounding tissue must be considered in formulation of an adequate model for prediction of the requirements for tumor destruction.

Power and power density, and energy density are not mentioned in the development of the model for tumor destruction. Since previous studies (25) had shown that power (and power density) is of importance in the interaction of laser radiation with biological systems, these parameters should be considered in the formulation of an adequate model. Based on relatively few tumors destroyed (54 tumors) at various energy levels it is concluded that "it is evident there is tendency for the ruby laser to destroy a tumor more effectively than the neodymium laser source". Since the tumors were located at some depth below the skin, it is necessary to take into account such factors as reflection from the surface and interfaces, absorption and scattering within superficial tissue layers and refractive indices at 6943 Å and 10600 Å respectively to provide a basis for the above statement. In regard to the graph comparing the energy absorbed at the ruby and neodymium wavelengths by the tissue suspensions, no information is provided as to how these measurements were made, except that a DK-2 spectrophotometer was used. If spectrophotometric determinations were carried out in the usual manner, measurements of transmission rather than absorption were made. Since a high degree of scatter is produced by cellular homogenates, no direct relationship

exists between transmission and absorption measurements. Furthermore spectrophotometric measurements on an essentially linear time invariant system at low power levels cannot be extrapolated to absorption of radiation at high power densities, as provided by lasers, by time varying systems in which phase transformations are produced.

It is probable that the temperatures quoted were due to direct irradiation of the thermocouples. This problem is discussed in the paper by Nowak et al (26). Minton et al (22) state that the temperature elevation, as measured by the thermocouple persisted for less than 1 millisecond. The established data for thermal conductivity and heat capacity of tissue indicate that the temperature elevations of tissues would persist for considerably longer periods. The rapid decay in the temperature elevation would therefore probably be due to temperature changes limited to the thermocouples. It is therefore likely that the temperature of the tissue in the region of the thermocouple junction was considerably lower than stated. It cannot be concluded, therefore, that evidence for phase transformation was presented.

The authors (27) suggest that the depth of the penetration of the laser beam can be controlled more effectively than that of either X- or gamma radiation. Factors such as scattering and absorption of energy by tissues at the various wavelengths require consideration in this respect.

The depth to which the tumor has extended requires further consideration. The extent of a tumor in the animal or in man is frequently difficult to determine, particularly if penetration into the soft tissues or into a body cavity has taken place. The depth and total volume of the tumor have to be taken into account, as well as the cross section, which was considered by the authors in order to establish a relationship between the energy required and tumor destruction. Predictions of tumoricidal effects is attempted (23)

on the basis of the nature of the immediate reaction of the tumor to the radiation. The conclusions were that completely flattened tumors following irradiation would regress, but tumors in which a crater had been produced would not regress. In agreement with others (4), Minton et al (23) did not find that survival of irradiated tumor-bearing animals was increased when the tumor did not regress. In the paper "The Laser, a Unique Oncolytic Entity" (28), previous studies on experimental tumors are reviewed.

In an investigation entitled, "A Comparison of the Effect of Microsecond and Nanosecond Ruby Laser Radiation on Rat Tissue and Mouse Melanoma: A Preliminary Report" (29), Minton and Ketcham studied the effect of ruby laser radiation on normal rat tissue and mouse melanoma at pulse durationso of the order of several hundred microseconds non-Q-switched and 100 nanoseconds Q-switched. The maximum non-Q-switched energy of the unit was 30 joules, which was probably the non-Q-switched energy used in the studies. The Q-switched energy used was probably of the order of 1 joule. Normal rat tissue and S_{91} melanoma-bearing mice were irradiated. The effects on skin, exposed liver and intrahepatic tumor implants were studied.

The non-Q-switched irradiation focused deep to the skin into the melanoma proper produced perforation of the skin and partial destruction of the underlying melanoma. The focused Q-switched laser beam produced no apparent effects on the overlying skin. These differences may have been due to the differences in irradiation energies. Seven days following irradiation, microscopic examination revealed coagulation necrosis in the tumor, but no skin changes following Q-switched irradiation.

The microscopic sections discussed were obtained five and seven days, respectively, following irradiation. Since this is ample time for partial or complete regeneration of damaged epithelial tissue, examination of sections

at earlier stages in regard to lack of damage to epithelium is indicated. Little tissue destruction was observed on single irradiation of two rat livers, non-Q-switched. Microscopic sections taken through the liver showed cellular cloudy swelling. No effects were observed grossly or microscopically on irradiation of the muscles of the abdominal wall. Single Q-switched laser irradiation of two rat livers resulted in defects. None were observed on muscle tissue irradiation.

Exposure to single pulses of non-Q-switched radiation of surgically exposed S_{91} melanoma liver implants resulted in partial tumor destruction. The effects of Q-switched laser radiation appeared to differ from the non-Q-switched irradiation. Defects were produced in the tumor mass, similar to those in the liver, and repeated Q-switched exposure was capable of "completely disintegrating small tumor implants". If the irradiated tumor implants in the liver were small, repeated unfocused irradiation would have been expected to injure normal surrounding liver tissue. Information regarding the surrounding liver tissue, or focusing of the beam would therefore be of interest. Repeated non-Q-switched irradiation of tumor implants was not attempted. The relative size and geometry of the tumor implants is significant; the relative size and geometry of the tumor implants exposed to the non-Q-switched and Q-switched irradiation was not discussed. Further studies appear warranted regarding the subsequent course of the small tumor implants, which had undergone disintegration.

The use of fiber optics for destruction of selected primary tumors and metastatic tumor implants is mentioned. That the decreased effect on the melanoma beneath the skin on nanosecond laser irradiation "may be due to a decrease in the velocity of the laser beam as it passes through the tissue" is not understood by the reviewers.

Since these studies were preliminary an attempt was not made to keep energy (one of the parameters) constant. In order to compare the effects due to varying peak power levels, it would be necessary to keep the other various parameters constant. Further studies on larger numbers of animals are warranted.

Attempts at utilizing animal tumors for models for the quantitation of energy requirements in regard to destruction of human tumors on the basis of laser wavelengths absorption of human and animal tumors respectively were presented by Minton (30). The author indicates that the equations presented for the prediction of energy requirements for destruction of experimental tumors may be applicable to human tumors. These equations are based on spectrographic determinations carried out on homogenates. Problems associated with spectrographic studies on tissue homogenates are discussed above. Several biological factors in addition to those discussed by the author would warrant consideration in the development of equations for the prediction of the energy requirements necessary for tumor destruction. The absorption of energy at a given wavelength by a tissue or tumor homogenate would be different from the absorption of the same amount of energy at that wavelength by an organized cellular system. Reflection, scattering, diffaction, refraction and other factors affecting the interaction with the primary radiation would be dependent upon the properties and anatomical location of the various tissue components. The characteristics of a highly complex cellular system such as tumor or normal tissues which require consideration include a large number of surfaces of different properties such as cell membranes, nuclear membranes, cytoplasmic constituents and subcellular organelles as well as differences between various structures such as blood vessels, connective tissue fibers, interfaces between different tissue layers and between cells within the same tissue layers. Similar considerations

pertain to secondary effects which are known to be generated as a result of the interaction of the primary radiation with the tissues. Thus, the dissipation and transmission of the various forms of energy associated with heat, pressure, acoustic waves, secondary electromagnetic radiation and particular matter, (charged or uncharged) would differ in an organized tissue system as compared to a homogenated suspension. The extrapolation from a single parameter to a number of unrelated parameters as represented by the absorption properties of a homogenate and interaction with an organized tissue system, respectively, consequently, does not seem to be justified.

Further considerations inherent to the problem of relating information obtained from homogenates to intact tissue or animal systems, have to be considered. These in essence are the multiple interrelations which are thought to bear on a specific biological system within the intact host, which cannot be reproduced in the experimental conditions imposed on an in vitro system. In particular regard to the study of tumors, these involve the blood supply to the tumor, the anatomical location of the tumor, and the relationship of the tumor to the host.

Discussion

The observations presented in the various studies on experimental tumors provide some orientation for further investigation of the interactions of laser radiation with biologic systems. Definite conclusions can not be drawn from this data which is preliminary for several reasons. The numbers of animals in the several groups studied so far is too small for meaningful statistical analysis. Laser devices are still in developmental stages; therefore, the practical difficulties of reproducing radi-

ation qualities coincident with specific stages of tumor development are considerable. Equipment and procedures have not been adequately developed for measurement of the various parameters of the interaction of laser radiation with tissues. Studies in normal animals which may serve as a basis for further investigation of interactions of laser radiation with normal and neoplastic tissues indicate the considerable complexity of the interaction of the incident primary radiation with the surface and with deeper structures

Irradiation of the intact skin overlying the tumor resulted in a plume of backscattered material and radiation not confined to the qualities of the primary radiation as had been previously observed in normal animals (3,8), and was subsequently studied in further detail.

The gross and microscopic examination of early and delayed effects of laser radiation on transplanted tumors suggested that some neoplastic tissue may be more suitable than normal tissues for the study of several biologic factors. The interaction of the radiation varies with different tissues as shown in normal animals (3,4,6,7,8,11,25). It was observed after irradiation of the intact abdominal wall that the reaction did not appear uniform hroughout the affected region. There was obvious severe damage to the skin while changes in the subcutaneous tissue appeared to be less marked. The underlying muscle was severely affected. Extensive hemorrhages occurred in and around the muscle, in the mesentery, and in affected areas of intestine. but free blood was not seen in the peritoneal cavity. Hemorrhage was not seen in the liver after irradiation through the abdominal wall, although subcapsular areas of necrosis several millimeters in diameter occurred frequently.

Some of the variables, which are inherent to the succession of tissue layers within the area involved by the primary interaction or secondary effects, are eliminated in neoplastic tissue. It may therefore be possible to investigate such factors as attenuation with distance from the site of primary interaction more adequately in neoplastic tissues than in normal structures.

The interactions of laser irradiation with cell populations of normal and neoplastic tissue were different in several respects. In relatively homogeneous normal tissues such as liver, the initially induced lesion did not appear to increase in size and was not followed by secondary necrosis of other parts of the same tissue or of adjacent structures. Repair became evident within 36 hours. Necrosis after irradiation of some tumors was progressive, proceeded usually to liquefaction, and involved tumor that had probably been outside the track of the primary radiation. It is however difficult to exclude the role of infection in ulcerated tumors.

The reactions of neoplastic and normal tissues to laser irradiation were similar in that early lesions were sharply demarcated from the non-irradiated adjacent areas, the architecture and outlines of cellular components were partially retained, the area immediately affected was not determined by the blood supply, and interaction with pigmented structures (i.e., melanin-containing tissue, muscle, liver, and highly vascular organs) was more marked.

In view of the differences in the reactions of normal and neoplastic cell populations, several types of tumors were investigated. Melanoma was selected to study the effects of interaction with pigment

over larger areas than was possible in normal animals in which melanin is confined to very small areas. Cloudman S-91 melanoma was included to study the effects of irradiation on rates and extent of metastatic dissemination. Osteogenic sarcoma and bladder carcinoma were selected for studies of interactions with cell populations of connective tissue and epithelial origin, respectively. The data, if survival of tumor-bearing animals is used as a criterion, suggested differences in the interactions of the various types of tumor with laser irradiation. The information available at present is insufficient to relate these differences to specific biologic characteristics.

Some relations of the parameters of laser radiation to the interaction with neoplastic cells are apparent. The total energy of the radiation is an important factor. The rate at which the energy is delivered is also important. In studies on Harding-Passey melanoma, regressions were not observed with single pulses in the 30-60 joule range. Radiation at a nominal output of 350 joules or more per pulse, delivered as single pulses, was followed by a considerable incidence of regression. Radiation at 6943 Å and 10600 Å appeared to have similar effects on regression of Harding-Passey melanoma at single exposures of 900 joules or more per pulse. Tumors of small size (10 mm. or less in diameter) showed a considerably higher incidence of regression than tumors with larger cross-sections. Important factors in determining the effects of the interaction include energy density and tumor parameters, in addition to the energy per pulse, peak power, and the total energy delivered.

Delivery of total energy from 350 joules to more than 2,000 joules as 30-50 pulses at intervals of 1 second (high pulse repetition

frequency) resulted in severe trauma followed by death in less than 24 hours in the majority of normal and tumor-bearing animals. Death appeared to be associated with severe lesions to adjacent normal structures rather than to effects on or of the tumor. It had been observed (4,14) that the effects of irradiation do not appear quantitatively similar when the same amount of energy is delivered at pulse-repetition frequencies in excess of 1 pulse/second as compared with delivery of the total energy as a single pulse of one millisecond duration.

Some regressions were observed with total energies over 150 joules delivered in doses of approximately 40 joules fractionated at intervals of 3-5 days. It appears from these observations (4) that the frequency at which successive pulses are delivered is significant in addition to the total energy and energy density. It had previously been observed (10) that the power levels and power density are of considerable importance in the interaction of laser radiation with normal and neoplastic tissues.

Averaging the survival time of irradiated tumor bearing animals in which complete regression was not observed, indicated reduction in the expected survival time as compared to non-irradiated tumor-bearing controls. However, this must be qualified by the difficulties of disassociating the effects of radiation on the general state of the animal from those on the tumor. Early fatalities usually followed laser irradiation of animals with large, far-advanced tumors. The reaction to injury induced by radiation, and consequent exposure of considerable areas to infection, would be poorly tolerated by a debilitated animal. Irradiation effects on other organs, particularly the intestine, may have contributed to the increased death rate. Lesions of internal

organs of an extent similar to those occurring in tumor-bearing animals were however well tolerated by normal mice. The pressure wave produced by laser irradiation, furthermore, may result in dissemination of residual viable tumor cells by direct extension or by metastasis through the blood and lymph.

The lack of regression following irradiation of animals with S-91 melanoma and of Lewis bladder carcinoma observed in one group of studies (4) may have been due to the low levels of energy per pulse available at the time of studying these tumors. In these studies, the animals with Lewis bladder carcinoma and with S-91 melanoma were irradiated furthermore at a relatively advanced stage of tumor development. The studies by Minton and Ketcham (21,22) on S-91 melanoma-bearing mice were carried out with radiation at considerably higher levels of energy density and at earlier stages of tumor development than those of Klein, Fine et al (4). The differences in the characteristics of the radiation and the state of the tumors probably account for the high incidence of regression of S-91 melanoma observed by Minton and Ketcham (22).

The microscopic appearance of specimens taken from the sites at which regression of Harding-Passey melanoma (4) had taken place suggested that tumor had been present. The presence of melanin in the histiocytes was compatible with the preceeding presence of melanoma in Swiss (white) mice. The regression of osteogenic sarcoma (Ridgway) induced by laser radiation was also followed by protracted chronic inflammatory changes, including histiocytes containing hemcsiderin. It is of interest that intact epidermal appendages are present in close proximity to the sites of chronic inflammatory changes associated with the sequelae of tumor

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BIOLOGICAL EFFECTS OF LASER RADIATION. VOLUME I. REVIEW OF THE --ETC(U)
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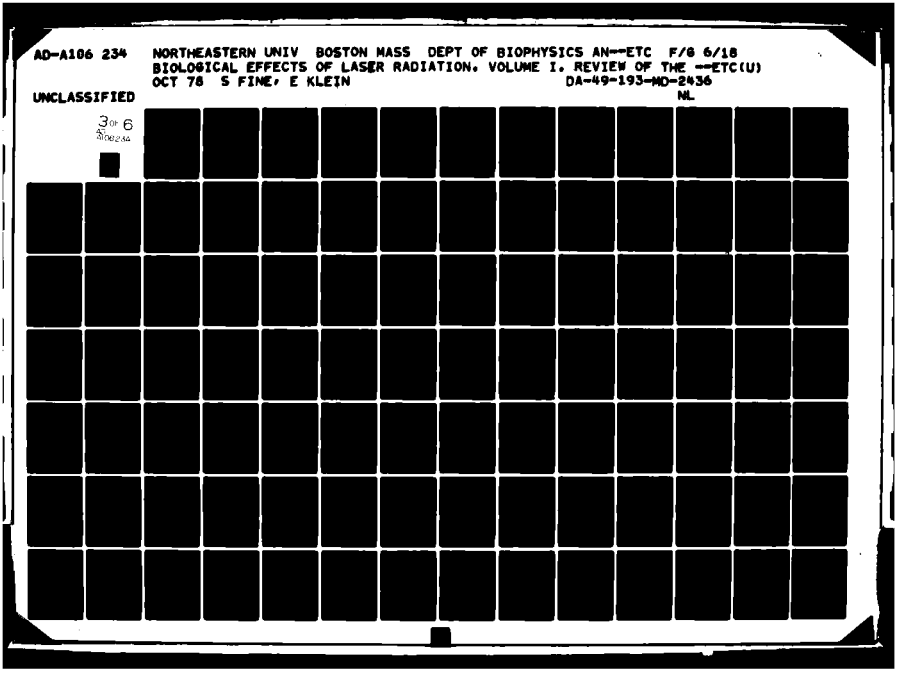

regression. The continued chronic inflammation and epidermal hyperplasia in some animals several months after the gross appearance had suggested complete healing, however, requires consideration of the possibility of delayed effects of the laser radiation (4). In the studies by McGuff et al (18) residual tumor cells were not found on excisional biopsy following laser irradiation of a number of experimental tumors. These authors do not indicate whether other histological changes were present.

Neoplastic changes attributable to laser irradiation have not been observed so far in normal or tumor-bearing animals in which regression permitted survival for protracted periods of observation. The observation of possible production of free radicals by laser irradiation in normal tissues (31,32) requires further study. Initial studies of the relationship of free-radical production and laser irradiation were inconclusive in Harding-Passey melanoma, since appreciable levels of free radicals were found in the tumor prior to, as well as after, laser irradiation.

Experimental studies of the interactions of laser radiation with transplanted tumors indicate that neoplastic cell populations have properties that warrant the use of tumors in future investigations of the interactions of laser radiation with tissue. The propensity for growth of residual viable cells, the consequent ease of end-point determination, the presence of pigment in some cell lines, and a high degree of vascularization facilitate the investigation of the differential effects of laser radiation in biologic systems. Considerable additional studies in experimental tumors are required to elucidate mechanisms of interactions of laser radiation with neoplastic cell populations. This

information will provide a basis for establishing indications and contra-indications to guide clinical investigation of laser radiation in malignant disease.

REFERENCES

1. Fine, S., Klein, E., Scott, R., Seed, R., Laser Radiation in the Syrain Hamster, Skin 2:43, 1963.

2. Klein, E., Fine, S., Scott, R., Farber, S., Observations on Laser Irradiation of Experimental Tumors, Proc. Am. Assoc. Cancer Res., 1964.

3. Fine, S., Klein, E., Scott, R., Laser Irradiation of Biological Systems, I.E.E.E. Spectrum 1:81, 1964.

4. Fine, S., Klein, E., Nowak, W., Scott, R., Laor, Y., Simpson, L., Crissey, J., Donoghue, J., Derr, V., Interaction of Laser Radiation with Biological Systems. I. Studies on Interaction with Tissues, Fed. Proc. 24 (1), Pt 3, suppl. 14: S-35; 1965.

5. Klein, E., Fine, S., Laor, Y., Simpson, L., Ambrus, J., Richter, W., Smith, G.K., Aronson, C., Interaction of Laser Radiation with Biological systems. II. Experimental Tumors, Fed. Proc. 24 (1. Pt. 3) suppl. 14: S-143, 1965.

6. Klein, E., and S. Fine, The Effects of Laser Radiation on Experimental Tumors, Ann. Meeting Am. Acad. Dermatol., Chicago, 1964.

7. Klein, E., and S. Fine, The Biological Aspects of Laser Radiation, Absts. 149th Meeting Am. Chem. Soc., Detroit, Mich., April 1965, p. 14.

8. Fine, S., and E. Klein, Biological Effects of Laser Radiation, Advan. Biol. Med. Phys. 1965 (in press).

9. Minton, J., Absorption Capabilities of Experimental Malignant Tumors, Life Sciences 3:1007, 1964.

10. E. Klein, et.al. (in preparation).

11. Seed, R.G., S. Fine, and E. Klein, Biological Effects of Laser Radiation, Boston Laser Conf., 2nd, 1963.

12. McGuff, P., Bushnell, D., Soroff, H., Deterling, R.,Jr., Studies of Surgical Applications of Laser, Surg. Forum 14, 143, 1963.

13. McGuff, P.E., Deterling, R., Levy, C.K., Bushnell, D., Roeber, F., A comparative study of the effects of Laser and/or X-ray Radiation therapy on experimental adenocarcinoma, Boston Laser Conf. 3rd, Boston, Mass., 1964.

14. McGuff, P., Deterling, R., Jr., Levy, C., Bushnell, D., Roeber, F., A comparative study of the effects of Laser and/or X-ray Radiation therapy on experimental adenocarcinoma, Boston Laser Conf., 3rd Boston, Mass., 1964.

15. Rounds, D.E., Olson, R.S., Johnson, R.M., The Application of the Laser to Cytology, Proc. Ann. Biomedical Laser Conf., 1st, Boston, Mass., June 1965.

16. McGuff, P., Effects of Laser R diaton on Tumor Transplants, Fed. Proc. 24 (1, pt. 3) suppl. 14: S-150, 1965.

17. McGuff, R., Deterling, D., Jr., Gottleib, L., Bushnell, D., Roeber,F., Laser Radiation of Malignancies, Ann. N.Y. Acad. Sci. 122:747-757, 1965.

18. McGuff, R., Deterling, R., Gottlieb, L., Fahimi, D., Bushnell, D., Surgical Applications of Laser, Annal. of Surg. 160 4:765, 1964.

19. McGuff, P., Deterling, R., Gottlieb, L., Gahimi, D., Bushnell, D., Koeber, F., The laser treatment of experimental malignant tumors, Canad. Med. Assoc. J. 91, 1089, 1964.

20. McGuff, P., Comparative effects of laser and/or ionizing radiation on experimental malignant tumors with report of synergistic tumoricidial effect of combined laser and ionizing radiation, Ann. Biomedical Laser Conf., 1st., Boston, Mass., June 1965.

21. Minton, J., Ketcham, A., Dearman, J., Tumoricidal Factor in Laser Radiation, Surg. Forum 15:335, 1964.

22. Minton J., Some factors affecting tumor response after laser radiation, Fed. Proc. 24 (1. pt.3) suppl. 14: S-155, 1965.

23. Minton, J., Ketcham, A., Dearman, J., McNight, W., The effect of neodymium laser radiation on two experimental malignant tumor systems, Surg.,,Gyn, Obst., 120:481, 1965.

24. Klein, E., S. Fine, E, Cohen, J. Ambrus, E. Neter, R. Lyman, R. Scott, Effects of Laser Radiation on Biological Systems, presented at American College of Physicians Meeting, Atlantic City, N.J., April 1964.

25. Fine, S., Maiman, T., Klein, E., Scott, R., Biological effects of high peak power radiation, Life Science 3:209, 1964.

26. Nowak, W.B., S. Fine, E. Klein, K. Hergenrother, W.P. Hansen, On the use of Thermocouples for Temperature Measurement during Laser Irradiation, Life Sciences 3: 1481, 1964.

27. Minton, J., Ketcham, A., The effect of ruby laser radiation on the Cloudman S-91 melanoma in the DCBA/2F, Hybrid mouse, Cancer 17: 1305, 1964.

28. Minton, J., Ketcham, A., The laser, a unique oncolytic entity, Am. J. Surg. 108:843, 1964.

29. Minton, J., Ketcham, A., A comparison of the effect of microsecond and nanosecond ruby laser radiation on rat tissues and mouse melanoma: A preliminary report, J. Surg. Res. 4:281, 1964.

30. Minton, J., Pulsed laser - an adjunct to cancer surgery, Ann. Biomedical Laser Conf., 1st, Boston, Mass., June 1965.

31. Derr, N.E., E. Klein, S. Fine, Presence of Free Radicals in Laser Irradiated Biological Specimens by Electron Spin Resonance, Appl. Optics 3:786, 1964.

32. Derr, N.E., E. Klein, S. Fine, Free Radical Occurrence in some Laser Irradiated Biologic Materials, Fed. Proc. 24 (1 pt. 3) Suppl 14:599 S-103, 1965.

CLINICAL STUDIES

This section deals with studies on humans other than those on ophthalmology and dentistry which are reviewed elsewhere in this report. Litwin and Glew (1) summarized the potential applications of laser technology to clinical medicine, such as the possible use of fiber optics, the application of laser devices to gross and microsurgical procedures and the exploitation of effects at specific wavelengths. Malt and Townes (2) have discussed clinical exploration of laser radiation. Fine, Klein, and Scott (3) reviewed early experimental findings in normal and tumor-bearing animals as a basis for orientating eventual clinical studies. The possible hazards were discussed. The authors emphasize that studies on the long term effects of laser radiation in animals and considerable additional understanding of the mechanisms of interactions are needed as prerequisites for clinical investigation. The exploration of laser radiation in the management of malignant disease was also discussed by Ketcham and Minton (4).

Studies on tumors in animals were oriented towards establishing guidelines for determining the conditions required for management of tumors in patients. Devitalization of the tumor with multiple appropriately placed laser pulses prior to removal of part of an organ which had been replaced by tumor was suggested. The possibility of removal of malignant tissue by laser radiation as a palliative measure, when definitive cure is not possible, was discussed. These

general considerations (1, 2, 3, 4) were based on animal and in vitro investigations rather than on direct experience with clinical studies on laser radiation.

Most of the published reports dealing directly with laser irradiation of humans would be benefited by documentation of the physical and, frequently, the biological principles involved. To some extent this may have been due to the lack of participation in clinical studies by a physical scientist with experience in biological research. Quantitation and interpretation of such results as were obtained, were consequently lacking. This, together with the fact that only preliminary comparative animal studies had been carried out, makes it difficult for other investigators to derive maximum benefit from the investigation in order to orient their own studies in man.

Considerations of inadequate background information also applied to patients with malignant tumors, who were irradiated. Although exploratory laser irradiation of inoperable terminal cancer may be justified by appropriate indications, adequate definitions of these indications for laser irradiation requires serious consideration. A questionable basis for studies in man may be presented by malignant tumors excessively rich in melanin which absorbs electromagnetic radiation over wide ranges of the spectrum. Since destruction of vascular integrity by laser radiation may also account for tumor regression, some rationale can be developed for irradiation of highly vascular tumors, such as hemorrhagic sarcomas. However,

these types of tumors are available as experimental neoplasms in animals and should be more extensively investigated before studies in man are initiated. These considerations are even more applicable to studies on tumors in man for which adequate routine management (with cure rates of 98-100%) is available (i.e., basal cell carcinoma, squamous cell carcinoma in situ). Obviously the exploration of benign diseases, such as a number of skin diseases including benign tumors, should also await a full knowledge of the effects which laser radiation can cause on a long term as well as on a short term basis. Furthermore, specific clinical indications for investigating laser irradiation in benign diseases in man have not been presented. Specific indications have not been adduced as yet which would lead one to expect a sufficiently favorable, unique response or important information, not otherwise obtainable to be demonstrable, which would justify taking risks in volunteers or patients with relatively inconsequential diseases.

A number of the reports on laser irradiation of man are largely inconclusive. In general the observations are too few in numbers to be suitable for objective or statistical evaluation. They consist almost entirely of case reports on very few patients with different diseases. Generalized conclusions, therefore, cannot be drawn from these reports, on which further studies in man could be based. A number of the disease entities (i.e., psoriasis, melanoma) which were studied have spontaneously variable courses thus rendering any interpretation of the finding difficult.

In general, it has been postulated that laser radiation has "selective" action on human tumors, presumably as compared to normal tissues, for which evidence is not available. Some of the biological

characteristics which are more prominent in certain types of tumor (such as pigment in melanoma or a rich blood supply in Kaposi's sarcoma), may be responsible for differential interactions and subsequent course. No evidence, however, has been presented that malignant cells per se differ from normal cells in their respective reactions to laser radiation. An intensive investigation of the reactions of normal and malignant cell populations to laser irradiation would be required for demonstrating that a difference exists.

Minimal damage to normal structures in the presence of total destruction of tumors was reported. These statements were based on observations obtained following irradiation of the tumor, (which was consequently damaged), while normal surrounding tissues, which had not been irradiated were not damaged. Direct irradiation of normal skin and other tissues has been shown to result in injuries to them (5).

Only limited consideration was given to the fact that tumors spontaneously undergo partial necrosis. Any traumatic agent can induce necrosis more readily in a tumor, which is about to exceed its precarious blood supply, than in normal tissues, which usually have a more adequate circulatory reserve. While there is general agreement that only superficially located tumors would be amenable to laser irradiation, intimations are presented that widely metastatic cancer may eventually be benefited. Unsubstantiated speculations concerning the mechanism of tumor destruction were entertained, such as: the induction of auto-immune processes (sometimes considered to be specific for a hypothetic tumor protein which has become anti-

genic); the selective alterations of an enzyme specifically essential for the tumor, (which has not been demonstrated in oncological studies), or, the release of cytotoxic agents from damaged tumor, which in turn are selectively toxic to the tumor and not to normal tissue (such a substance has not been demonstrated).

In some cases, it is difficult to follow or to determine the sequence expected to underlie the presentation. Methodology is at times inadequately presented, which would prevent repetition and, therefore, confirmation by other laboratories; the sections on results usually include references to future plans or incomplete studies in progress, which are then referred to (in subsequent reports) as though they had been authenticated data. However, it must be recognized that several of the reports are considered by the authors as preliminary, and, in some cases, were carried out with prototype laser units which were, at that time, not highly reliable insofar as physical parameters are concerned. Since these were the only units available at that time, extensive measurement of physical parameters was not feasible.

In the light of the nature of some of the investigative material, which should be considered as exploratory, the evaluation given below could not be presented in as critical a manner as was considered desirable in other sections of this report.

Studies on both Caucasian and Negro volunteers were presented by Goldman (6). Negro skin apparently showed minimal lesions, the induction of which was accompanied by a slight pricking sensation at a level presumed to be 0.5 joule per pulse at 6943 Å. At 5 joules per pulse more extensive changes were noted in Negro skin. No lesions are reported to have been detectable under similar conditions of exposure in Caucasian skin. Various attempts were made to increase the absorption of radiation by the skin, including stripping of the keratin layer, topical application of soot obtained from a candle, and application of the dihydroxy acetone to Caucasian skin. The use of dihydroxy acetone presents a useful approach to relate effects to the degree of pigmentation. However, studies to indicate that absorption of the radiation by keratin is significant in relation to stripping would be of interest. Indeed, the presence of a relatively transparent superficial layer may, in some cases, result in increased injury to the underlying absorbing layer.

In the presence of increased color content a clear white zone developed in skin following laser radiation (presumably at both 0.5 joule and 5.0 joules per pulse). The reaction is reported to have been more intense in the skin of Negro volunteers. Dark hair was charred. Irradiation was accompanied by a "puff of smoke". This may have been associated with backscattered material at a velocity such that it would at present be considered a plume. A patient with argyria did not appear to react to laser radiation. Reference was

also made to a variety of studies in progress such as burns produced in baso-squamous papillomas and studies of the effects of laser irradiation on red cells, capillaries, old hemorrhage in the skin, pigmented nevi, pigmented basal cell carcinoma, tattoo marks, excised flaps of human and animal skin, and callosities colored with various dyes. Data on these studies as to the methods which were used, the number of samples studied, the directions which the proposed studies were taking, and what their objectives were would have been of interest. Microscope lenses were damaged by laser radiation of the characteristics referred to above (presumably 5 joules per pulse, with a pulse duration of the order of milliseconds). Although, as suggested by the authors, the coating or the cement may contribute to damage, interposition of a cover slip would eliminate backscattered material as a cause for some lens damage. Attenuation of the energy by these lenses on irradiation of tissues require evaluation since this would affect the energy delivered to the tissue. The authors also indicate that measurements of red and infrared reflection and transmission are being made without indicating how these measurements are being carried out, nor what the result of the measurements are. Measurement of these parameters at the power levels and geometry used is important. Further references are made to experiments which had been carried out on fiberoptics "combined with laser beams" to provide deeper penetration. It would be of interest to know how this combination was achieved, and what the relative effects with and without transmission through fiberoptics were. Although preliminary studies were carried out by the reviewers and their associates in 1962 on transmission of laser radiation through fiber optics bundles the relatively poor results at that time indi-

cated that considerable thought would have to be given to bundle coherency and damage. Considerable effort has been expended in the past several years in attempting to couple lasers with fiber optics systems. (25) Some biological studies were reported (26).

Microscopic examination revealed a superficial ulcer and inflamatory exudate in laser induced lesions; the histochemical studies failed to show changes in the sulfide or disulfide groups of the keratin layers. It must be concluded that the lack of changes in the sulphydryl group, however, does not indicate that the keratin layer was not damaged, but that the sulfur containing amino acids do not participate in the interaction.

Goldman et al (7) further reported cytological studies on cutaneous cells and on blood cells. In the section headed "Techniques" of this publication the authors indicate that they were also studying fungi and bacteria in various media and containers. Although the nominal energy outputs of the various lasers used are given, the characteristics of the radiation (such as energy and energy density) are not well defined. To some of the cutaneous and blood cell preparations various dyes were added (Eriochome black T, Evans blue, Trypan blue). Some of the cell preparations were also stained by routine histological techniques and some were examined in the unstained state after they had been fixed and embedded in paraffin.

The objectives of these experiments are not readily apparent. The authors state that hemorrhage in vivo as a result of laser irradiation presents a hazard, and therefore effects on stained red blood cells were investigated. The relationship of effects on stained or

paraffin embedded preparations to investigations in vivo is not discussed. The cutaneous cells included keratinizing and non-keratinizing cells of the epidermis and cells from various disorders such as non-pigmented basal cell carcinomas, warts and molluscum contagiosum. Epithelial cells included scrapings from the buccal and vaginal mucosa and Hela cells obtained from tissue culture preparations. While there was no recognizable effect reported in these preparations as such, complete disintegration was observed when the cells were vitally stained. The debris was reported not to be susceptible to counter staining. Description or details to indicate more clearly the results of laser radiation, whether any differences between these various types of tissue were apparent or whether any indication of specific significance were obtained would have been desirable. Varying the concentrations of the dyes to determine whether different thres hold levels for selective destruction of various cellular components or subcellular organelles exist would have been of interest. Exfoliated cells of ulcerated melanoma showed no melanin granules following irradiation. Nuclear structure however appears to have been preserved. On the other hand unstained material obtained from melanoma is reported to have undergone cellular fragmentation. It is difficult to reconcile these observations on melanoma cells. If nuclear structure was preserved, then cellular fragmentation could not have been complete. Sharply demarcated areas, indicating presumably the site of irradiation, were observed in paraffin sections of pigmented and non-pigmented basal cell carcinomas, warts, baso-squamous acanthomas and molluscum contagiosum. The significance of the observations is not discussed.

Defocusing resulted in spotty distribution of destruction and residual small islands of unaffected cytological material. It appears to the reviewers that these results may have been due to differences in the cytological material, but more probably are due to non-uniform distribution of energy within the beam.

Studies on blood smears are indicated, as previously stated, to be important because of injuries to blood vessels and blood cells following irradiation by "high intensity lasers" of rabbit ears and angiomas in man. It is difficult to determine whether the effects of laser radiation on blood smears can be extrapolated to the induction of hemorrhage. The effects on normal blood smears are described as a whitened area of disappearence of the red cells. A description of cytological changes associated with the effect observed by the authors as "an explosion with a formation of a superficial bomb crater" would have been of interest as it might have indicated effects other than heat per se. White cells were not damaged. This has been stated in a number of reports by other authors. Studies in progress on amoeboid movement and alkaline phosphatase of white cells are listed without indication of the methodology or results. Similar effects on the red cells and white cells, respectively, are reported on smears obtained from a patient with lymphatic leukemia. The reasons for this experiment are not apparent or stated. Buffy coats or purulent exudates were found to undergo undefined changes following laser irradiation. Dried preparations of cell aggregates obtained from a number of skin lesions are reported to show "a grossly visible area" marking the site of irradiation. Extensive cellular destruction was present. Moist preparations were significantly less damaged than dry pre-

parations. It would appear that this may have been due to the thermal properties of water. Vital staining of all materials significantly increased the degree of undefined destruction. If histological changes had been described, this might have provided information as to whether differential effects on cellular components occurred. Some explanation of this could have been derived from spectrographic studies.

The authors indicate that they consider "the most practical and simplest test for studying attempted protection of biological material against the laser beam" to be the use of hair preparations mounted on glass slides. It appears questionable that an essentially inactive structure such as hair would lend much information in regard to actively metabolizing systems. The effects on hair would be largely due to its pigment content, while most tissues do not contain significant or any amounts of melanin. It would therefore be difficult to extrapolate from the effects on pigmented material to non-pigmented structures. The differences between the low water content of hair and high water content of most tissues would also make comparison of the respective effects difficult. Furthermore the structure of hair is markedly different from that of most organized living tissues, which would prevent correlation of the respective interactions. It therefore appears that the differences between hair and the majority of other tissues are such that the hair cannot be considered as a model for the study of laser hazards. Further data with regard to the references made to detailed histochemical studies on hair following irradiation have not been described in the publications reviewed.

Although the authors had indicated that they were studying a number of fungi and bacteria, the only reference to micro-organisms appears to be to tinea versicolor. In the absence of Evans blue no changes were observed, while following vital staining, destruction of both the epidermal cells and fungal elements was seen. Chromosome preparations from "chromosome cultures", (not otherwise identified in regard to nature of origin or method of preparation) are reported to show no effect unless stained with orcein which resulted in extensive destruction following laser irradiation. There is no indication of what is meant by "destruction" in regard to histological or cytological descriptions.

It is difficult to be clear about the results observed, particularly since methodology and sources of materials are not adequately described. Presumably the objectives of the investigation were to generally screen the effects of laser radiation on a number of available preparations. The conclusion would appear to be that coloring which results in absorption of the radiation (expressed by the authors as "increased the energy of absorption of the laser beams") results in increased damage to cellular and sub-cellular elements.

The paper reviewed above was a short report. Since the number of samples studied in each case are not given, and since a large variety of materials were studied, it must be presumed that very few samples of each material were irradiated. The results obtained consequently, cannot be considered as of statistical significance.

Goldman et al (6) reported parallel studies on normal human skin and benign skin diseases. Injuries induced by laser radiation on colored skin were more pronounced than in white skin in regard to gross

and microscopic appearance of the lesions. Goldman et al(8,10) report the presence of acanthosis and bizzare nuclei which resemble carcinoma in situ in epidermal cells following in vivo laser irradiation in humans. Notwithstanding the observation of histological changes suggestive of formation of skin cancer following laser irradiation, studies on normal human volunteers and on patients with benign skin diseases were continued without intervening studies on animals.

No marked changes were found in psoriatic plaques following laser irradiation whether or not tar or dye had been applied to the lesions, in contradistinction to previous findings by the authors and others, in which the application of dyes were found to alter markedly the effects of laser radiation on skin. Angiomas in infants (33) and children, port wine angiomas and senile angiomas were also exposed to laser irradiation at 6943 Å. Angiomas in infants and older patients underwent resorption. Since angiomas in children have a high spontaneous regression rate, it is difficult to evaluate the extent to which laser irradiation contributed to their resolution, as compared to the natural course of the disease. It should be noted, however, that regression of angiomas as a spontaneous phenomenon occurs gradually while that induced by laser radiation presumably proceeded more rapidly. Obviously a great deal of additional studies on the safety of the procedure in regard to long-term hazards are indicated, before laser radiation can be considered as a possible approach to the treatment of a spontaneously resolving, benign, rather insignificant skin lesion in early life. Following observations on long term results, it will then be necessary to evaluate statistically the potential therapeutic advantages which laser irradiation may offer in comparison to established procedures in the management of this disease.

The vast majority of basal cell carcinomas and squamous cell carcinomas of the skin are amenable to management by established techniques, such as surgery (including electrosurgery and cryotherapy) and radiation therapy. By these methods the cure rate is in excess of 95%. Those types of cancers present serious therapeutic problems when they become deeply invasive (basal cell carcinoma) or metastatic (squamous cell carcinoma). Fortunately this is a rare occurrence, since the superficial lesions are usually recognized in time for definitive management. In view of the highly adequate established methods of managing primary epithelial tumors of the skin, it is difficult to assess the clinical indications for exploring laser radiation in these tumors. This becomes a matter of particular concern, since the harmful effects of laser radiation, particularly the long-term results, have not been adequately explored. Even from an immediate point of view, some possible dangers are apparent which require exclusion before laser radiation is applied to cancer which is curable by present methods. Possible immediate sources of danger have to be considered in view of our lack of knowledge of the mechanisms of interaction of laser radiation with tissues. The energy transformations and possible pressure waves associated with the initial interaction could result in the dislocation of viable tumor cells which may then be spread from the original site of the tumor by local extension into deeper layers of tissue. Alternately, dissemination of tumor cells may occur, if they are propelled by the sudden generation of pressure waves into the blood stream or the lymphatic channels from which they could be further disseminated. This could result in metastatic spread, earlier than would occur in the natural course of the disease.

The problem of local extension has to be considered in regard to basal cell carcinoma as well as squamous cell carcinoma. Local extension may result in a buried lesion which may proliferate without becoming apparent, until it is no longer curable. In regard to squamous cell carcinoma the induction of a metastatic process by blood or lymph born dissemination would obviously place this type of tumor beyond the reaches of curative procedures. Similar considerations apply to other types of primary cancer, such as malignant melanoma, which are well known for their propensity to disseminate as a result of superimposed trauma. To date, animal studies have not been conclusive in demonstrating whether laser irradiation of experimental tumors may result in an increase in the degree of local extension or in the rate of development of metastases. However, there is some indication that local extension can occur. It would clearly be indicated that pertinent data from experimental tumors in animals be obtained before clinical studies are undertaken. Since laser irradiation of primary cutaneous tumors in man are not based on any specifically unique indications, studies on otherwise curable tumors in man would appear inadvisable on the basis of inadequate knowledge of immediate as well as long-term hazards.

Initial studies by Goldman and associates (8) on laser irradiation of basal cell carcinoma failed to show eradication of the tumor. Residual tumor cells were found and early recurrence was reported. Goldman and Wilson (9) subsequently reported laser irradiation of 8 lesions in a patient with multiple nodular basal cell carcinomas at energy densities ranging from 20 to 18,000 joules per cm^2. One of these 8 lesions was irradiated with 4 successive pulses at 100 joules

per pulse at 6943 Å covering overlapping fields of 0.9 cm^2 which apparently covered the surface area of the tumor. This lesion appeared to regress while the other 7 irradiated lesions showed partial destruction but did not disappear during observation periods ranging from 1 to 5 months. In the skin adjacent to the site at which complete regression had been observed on excisional biopsy, a basal cell carcinoma appeared within 6 months of the irradiation.

It is difficult to know in a patient with multiple basal cell epithelioma who may continuously develop new lesions, whether the basal cell carcinoma found adjacent to the site of irradiation had arisen de novo or represented a recurrence. The possibility that its origin may be associated with laser irradiation can also not be excluded. This possibility should be kept in mind, particularly in view of the authors' findings of histological changes in cutaneous epithelium following laser irradiation which consisted of acanthosis and bizzare nuclei resembling carcinoma in situ(8,10). The authors rightly point out that no conclusions could be drawn from their studies since the number of lesions observed was too limited to permit evaluation of the data and the observation period was too brief to justify definitive conclusions. The authors, however, believe that the 8 tumors irradiated could be viewed as a control study for assessing various parameters of laser radiation (i.e., differences in energy density) and state that it was possible to observe therapeutic results. They refer to the effect on the tumor which had regressed as an "excellent therapeutic result" notwithstanding their own statement that neither statistical nor definitive biological significance could be assigned to these studies. They concluded

that their data indicate a relationship between the energy density and the eventual response of the tumor to irradiation. Documentations of this conclusion require further study at various power and energy levels. The authors state that although no significant epidermal or vascular changes were noted which would suggest precancerous changes in the skin or damage to the blood vessels, longer periods of observations are required to determine the late effects of laser radiation on the tissues of man. They further advocate that conventional methods of surgery and radiation should be used in the treatment of basal cell epitheliomas.

McGuff et al (11) reported studies on 3 patients with malignant melanoma and one on a patient with a squamous cell carcinoma of the orbit. The initial part of the paper is concerned with the history of the development of lasers and the physical aspects thereof. The statement that "the laser" has the unique characteristics of being coherent, monochromatic, and capable of high intensity should have been modified, since laser radiation, while it possesses a high degree of coherency and a relatively narrow bandwidth, is not completely coherent nor monochromatic. In conjunction with the studies on man, animal studies are reported. Malignant melanoma of human origin which had initially been grown in cheek pouches of hamsters, was transplanted beneath the mucous membrane of the cheek pouch of a second series of hamsters. Eighteen tumors of unstated size were irradiated, and 17 were used as controls. Amongst this group of tumors both amelanotic and melanotic tumors were found, although they were derived from a single source. Both the size and pigment content of the tumor on irradiation would be of significance since these factors may affect the results obtained. In the studies on thyroid carcinoma of human origin transplanted to the cheek pouch of the hamster, the size of the

tumors on irradiation is not given. It is presumed that serial histological sections were studied to ascertain that all tumor had been destroyed.

Following irradiation of malignant melanoma of undefined size, in mm at 360 joules, at 6943 Å, a few malignant cells were evident on histologic examination of one lesion 6 weeks after laser irradiation. Apparently other tumor nodules were irradiated, but not reported. Other tumors irradiated in mm include a recurrent squamous cell epithelioma of the orbit, an intranasal melanoma, and surgically exposed subcutaneous metastases of melanoma. Complete regression was not observed in any of the irradiated lesions.

The author's statement "it is likely that a more profound biological effect may be obtained with a laser of lower wavelength than that provided by ruby" would require substantiation. It is presumed that lower wave length means a shorter wave length.

Methods for measurement of temperature are described. A thermocouple probe was inserted within the tumor, and temperature measurements were made during and following irradiation. It is probable that some of the temperatures reported, such as 716°F at 2 mm., and 201°F at 5 mm., are due to direct irradiation of the thermocouple, and may not correspond to the actual temperature of the tissues at those depths. Time characteristics of the thermocouple response in tissue would give further information as to whether these temperatures were tissue temperatures or partly due to thermocouple radiation absorption. Since it is difficult to establish accurately the positioning of a probe, the depths given - 2 mm. and 5 mm. - must be considered as estimates. Although the so-called "thermal decay time" for a complete

tumor system during and following laser radiation was compared with thermal changes produced by cautery, certain problems arise in comparing these modes of injury, since the initial energy distribution within the tumor differs in both cases.

The discussion cannot be easily reviewed. The heading of the first part of the discussion is "Relationship of Laser Energy to Time and Relativity". Some personnal hazards which are discussed were previously described more extensively by various authors (5).

McGuff et al (11) state that the effect of laser energy on normal tissue is minimal and healing is rapid, but present no evidence to support this statement. This is in direct contradiction to the published findings (discussed in another section) of the various investigators who have intensively studied effects on normal tissues. The effects on normal tissue depend on the properties of the tissue and of the radiation. If the normal tissue, such as the hamster cheek pouch, is relatively transparent to the radiation, the effects will be limited to the blood vessels. However, if the tissue is relatively opaque to the radiation (such as pigmented skin or liver) then a definitive effect will be observed.

In discussing the effects of laser energy on tumors, the authors state (11) that the effectiveness appears to be related to several characteristics, including either energy density or power density. Both energy density and power density are of consequence (12). The total energy and the total power as are not considered as parameters, unless this is implied as the ratio of the dose and the target area to tumor size. It is stated that "laser has a minimal immediate effect" and "an intermediate effect of progressive regression" of the tumor.

The final effect is stated to be gross dissolution of the tumor, following which no viable tumor cells are observed on histologic examination. The basis for this generalization is obscure, since the authors, in agreement with other workers in the field, have observed numerous occasions when this sequence of events did not occur and viable tumor persisted after laser irradiation.

Residual tumor cells were found in all lesions exposed to laser irradiation (11). The authors indicate however, that substantial tumoricidal effects have been observed although total resolution of the tumor was not present. The authors suggest that the lack of total tumor resolution in man was due to inadequate dosage. The authors further indicate in the section on results that "certain" cardiovascular lesions, skin lesions and hemostasis are under study by them in patients. Data concerning results of these studies has not been found by the reviewers.

The authors (11) suggest a number of criteria for "laser use" referring to it as "the surgical application of laser" and state that certain "guidelines for its use have been established". These criteria include "treatment of deeper lesions, the tumor must be exposed to adequate approach" and "internal tumors accessible by endoscopic instrumentation or modified fiber optics applications should be suitable for laser treatment". Further study will be required to develop optical methods for transmitting adequate amounts of laser radiation to effect tissue destruction, since this is not possible by available light pipe or fiber optics equipment.

In their summary the authors (11) state that for "effective laser therapy" tumors must be exposed. Since tumor resolution was not observed in any of the patients treated and since these early attempts

cannot be construed as therapeutic, but have to be viewed as exploratory, it appears premature to refer to these procedures as effective therapeutic measures. The authors imply that laser radiation initiates a progressing process which then continues to result in tumor resolution. This intimation is made although, according to the authors' own observation (11), complete tumor resolution did not occur in any of the patients treated. The remainder of the summary infers the inactivation of a hypothetical enzyme necessary "for the metabolism of the tumor" which is postulated to undergo inactivation or change in specificity as a result of laser irradiation. Although "high electrical fields" may change molecular bonds, the presence of high electric field in tissue is difficult to establish.

Goldman (13) reported on the effects of laser radiation at 6943 Å and an energy output of 0.84 joules per pulse (pulse duration of the order of a millisecond) in a paper entitled, "Pathology of the Effect of the Laser Beam on the Skin". In this report gross and microscopic appearances of the lesions produced by laser radiation in Caucasian and Negro volunteers are reported in addition to a number of insects (black widow spider, cockroaches, caterpillars), smears from buccal and vaginal mucosa, pigmented and non-pigmented basal cell carcinomas, melanoma and other structures to which these authors refer in several other publications. No details of their methodology are given in this presentation. Goldman et al (13) report inflammatory changes in pigmented skin following laser irradiation which were not apparent in Caucasian skin similarly exposed. As described in another report by the authors (10), stripping of the keratin layer with and without application of

soot did not produce pathological changes at this energy level. In colored skin (presumably Negro skin rather than artificially colored) laser irradiation produced superficial lesions with fragmentation and splitting of the stratum corneum. In some lesions the cells of the upper layer were eosinophilic and pyknotic, indicating that they had undergone degeneration. Basal cells showed hydropic degeneration with decrease in cellular size, pyknotic nuclei and eosinophilic staining. There was considerable variation in the degree of injury at the cellular level. The area adjacent to the site of irradiation showed edema in the epithelial cells as well as in the dermis. A perivascular infiltrate was also present. The capillaries were dilated and the endothelial cells were distended. No changes were observed in the eccrine sweat glands at the lower level of the dermis. The authors do not report on changes in the melanin granules although one would expect that these would have been altered. Since the authors suggest that the difference in the reaction of colored and Caucasian skin respectively is due to the increased absorption of energy by the colored material, the changes in melanin in human skin would be of significance as one of the important distinctive histological effects.

In their observations on the effects of laser radiation on albino and black rabbit ears, the authors (13) indicate that loss of melanin pigment had occurred. Skin of the black rabbit had undergone destruction which extended through the dermis into the cartilage.

A deep reddish discolored zone appeared following irradiation of the head area of the caterpillar. Histologically, a sharply demarcated zone of coagulation necrosis was observed. The blackish-brown setae of

the caterpillar assumed a golden-yellow appearance following laser irradiation. The significance of this study is not clear.

Studies on experimental tumors in animals (5, 14) as well as normal animals (15, 16) have indicated that pigmented structures (melanin, hemoglobin) appeared to be differentially affected by laser radiation at 6943 Å and 10,600 Å, as compared to non-pigmented structures. Pigmented tumors in man, both benign (nevus, hemangioma) and malignant (malignant melanoma, Kaposi's hemorrhagic sarcoma) were included in clinical explorations of laser radiation in humans (8, 10, 11, 17, 18).

Goldman et al reported observations (8, 10, 19) on irradiation of benign nevi and malignant melanoma in patients. Some of the lesions were excised following irradiation while others were left in place for varying periods. The authors report a superficial coagulation necrosis distinguished from that produced with electrocoagulation or heat cauterization by the sharp line of demarcation, as previously reported in animal studies by Fine, Klein et al (3, 5, 14, 15, 16, 20). Goldman et al (19) further remarked on the resistance of hair follicles to laser radiation. In this report they state that tissue necrosis continues to spread for some time after irradiation. This reaction appears to differ from that reported by the authors (9) in irradiated basal cell carcinoma, in which delayed reactions were not observed. Reference is made in this report (19) to focussed radiation without stating the beam convergence, thus rendering it difficult to assess the distribution of the energy delivered in relation to the effects observed. Following an interval of 21 days after irradiation, micro-

scopic sections showed an atropic epidermis without abnormal cells and a sharply demarcated fibrotic scar. A description of the immediate specific changes, and of the subsequent course would have been of interest.

The observation for instance, that hair follicles are "resistant" to laser radiation is not supported by documentation. Insufficient documentation relating to gross as well as microscopic evidence was presented in regard to: whether hair growth continued; the degree of pigmentation of the hair; where the hair follicle was located in relation to the irradiation site, or in relation to lesion; what changes occurred in other tissues (normal or pathological) which did not occur in the hair follicle, thus indicating its "resistance"; whether "resistance" was indicated by an isolated observation, or was noted over a period of time, how many hair follicles were so observed; etc.

Similar considerations apply to the proposed central topics, such as nevi and melanomas, which were irradiated. Some of the important aspects of these studies require further documentation. Information regarding the lesions (i.e., size, shape) would permit these observations to be compared with other studies. The sequence of changes induced by laser radiation warrants consideration. Further studies are indicated to extend observations, which are presented, and to substantiate the interpretations. Reports on the follow-up studies over more protracted observation periods will provide additional information regarding these preliminary finding.

Irradiation of the malignant melanomas was reported (19) to have been studied in four patients (although only 3 patients are enumer-- ated. One of the 3 patients was apparently studied at two energy levels). Excised nodules of melanoma were irradiated . The nature of the destructive changes produced are not described. Red cells in the area of the irradiation are also reported to have absorbed "considerable laser energy" and to "show changes". Further information on the nature of the changes or the manner in which the absorption of energy was demonstrated, would be of interest.

In a second patient a melanoma which had developed from a lentigo maligna, was excised and irradiated with unfocussed and focussed radiation at a level given as 125 joules per pulse. The focussing characteristics of the lens system are not presented. Superficial destruction and deep necrosis were produced by the unfocussed radiation in this in vitro specimen. The effects of the focussed beam were stated as necrosis extending to approximately 4-5 mm. in depth. Whether the effects on the melanoma differed from those on normal structures would be of interest, as would a description of the gross and microscopic effects. The lesion, which was irradiated, apparently was a primary melanoma (there is no statement on whether it had extended). It would be of interest to determine whether and how the effects of laser radiation on primary melanoma differ from those on metastatic melanoma, in the one or more patients included in this report.

In a third patient, melanoma of the back was irradiated in vivo.

Both focussed and unfocussed radiation at a pulse level of 50 joules were employed (19). The lesion was excised within 24 hours following irradiation. It would be of interest to know whether this was a solitary lesion or whether at the time of irradiation metastatic disease was present. This would be of significance, since trauma to primary melanoma before or during surgery is contra-indicated, in order to avoid the possible dissemination of melanoma cells. Blood vessels in the area of irradiation were reported to have been thrombosed. Since the specimen, however, was obtained 24 hours following irradiation, it would not be possible to determine whether thrombosis occurred immediately or whether it arose at some time following irradiation as a secondary phenomena. In the latter event, there would have been enough time for viable cells of malignant melanoma to have entered the blood (or lymph) vessels and thus be able to initiate metastases. The force produced during irradiation dislodges cells and injures blood vessels (5). Thus the conditions are provided for possible dissemination of malignant cells. Animal studies are not conclusive but indicate that extension of malignant tumors may result, depending on the conditions of laser irradiation. It would therefore be of importance to state what precautions were taken to prevent the possibility of dissemination of malignant cells.

The patient listed as the fourth is stated to have the same number of metastatic nodules (i.e., 53) of melanoma as the patient listed as the first. Three nodules were exposed to "the ruby laser

and to the neodymium laser". It is not clear from this statement whether exposure was concurrent, successive, superimposed, or whether one or more nodules were exposed to only one type of radiation. Conceivably several different areas on each nodule were exposed. This aspect of the methodology, however, is not presented, making it difficult to interpret such description as is given in the observations. This consists of a statement that "deep necrotic zones" were produced by a ruby laser at 110 joules per pulse where the radiation was focussed. The effects produced by the neodymium laser (at an energy level of 42 joules per cm^2 obtained by focussing at 10 joule output), are described as having been "deep destructive changes". In the absence of a clear description of gross or histologic appearance it is not clear, whether there were any differences between the effects nor what these differences might have been. At a level of 10 joules or less "superficial" changes were observed.

In two patients suspected of having melanoma, laser irradiation was carried out. One of these patients was found to have angiokeratoma which was irradiated at 6943 Å with (presumably a single pulse) at 0.5 joules per pulse. This lesion was found to have undergone superficial changes. The nature of the gross or microscopic changes is not stated. In the second patient with suspected melanoma, the lesion was found to be a nodular hidradenoma which showed extensive destructive changes following irradiation at energy levels of 110 joules per pulse obtained from a ruby laser. Although a microscopic section was made to provide the diagnosis no data are presented on such microscopic changes as might have been produced by the radiation.

The authors state that they attempted to provide controls for laser experiments on living tissue by irradiating a tattoo mark on the forearm of presumably a normal individual in vivo at 100 joules. Radiation was unfocussed and presumably at 6943 Å. The epidermis in this individual was found to have been lifted by the impact. Extensive basophilic changes were noted along a "wide broad area". Dermabrasion prior to irradiation increased the area of destruction. The sizes of the areas involved by irradiation or the reaction to it, are not stated and therefore, can not be compared. Another portion of the tattoo was exposed to radiation at 10,600 Å at an energy density of 42 joules per cm^2, probably at 10 joules. Destruction was reported to be much deeper than that produced by the unfocussed radiation at 100 joules per pulse obtained from the ruby laser. Although these studies were carried out at different wavelengths, they were not carried out at similar energy levels nor at similar degrees of focussing and consequently do not provide a meaningful basis of comparison. This attempt to provide control studies for observations made on neoplastic lesions thus introduced further variables both in regard to the physical characteristics of the radiation and the biological characteristics of the system under study.

Reference is made to studies in progress with Q-switching techniques applied to ruby and neodymium lasers, respectively. It is stated that the areas of impact are smaller but that the destruction is deeper in some undefined tissues while in other the changes are

more superficial. These presumably refer to differences observed on non-Q-switched and Q-switched irradiation. It is probable that the Q-switched and non-Q-switched energies were not equal. The type of destructive changes, furthermore, are undefined. The statement is also made that preliminary experiments with "the laser of an exit energy of a 1000 joules" at an undefined wavelength have shown much more extensive changes in tissues, which however are undefined.

The authors further refer (19) to studies in progress on tumors in hamsters induced by polyoma virus and methylcholanthrene induced tumors in mice. Although the relationship of these tumor systems to pigmented tumors in man is not clear, the basis for these studies appears to relate to "changes in immunological mechanisms which may be produced by the effect of the laser beam which would be more specific for the tumor tissue". Destructive changes were reported in polyoma virus tumor at energy levels of 1200 joules at an undefined wavelength. Since only superficial changes were observed in methylcholanthrene induced tumors, the authors conclude that hyperkeratoses may exert a protective action, since hyperkeratotic masses were present in the methylcholanthrene induced tumors.

The authors conclude by stating that at the time of the study, the laser should be considered as a laboratory tool and not as a practical surgical instrument. They indicate that little pain was associated with the interaction.

Helsper et al (18) reported observations following irradiation of nevi and malignant melanoma in man. Energy levels varied from

0.1 joules to 2 joules per pulse focussed to a 0.25 mm at the target. Since the pulse duration was 1 millisecond at 6943 Å, the peak power output was probably less than the 20,000 watts given. The methods of estimating the target reflectivity as 40% and the loss through the lens as 6% is not given. In excised nevi a small area of necrosis appeared in the center of the lesion. Tissue destruction was observed to extend to the pigmented basal layer of the epithelium without apparent changes in the non pigmented supporting tissues.

Two patients with wide-spread metastatic malignant melanoma were irradiated. The subcutaneous nodules which were selected for irradiation were treated with adrenalin to prevent bleeding and with a local anaesthetic to avoid pain. The lesions were surgically exposed prior to irradiation. The number exposures varied from 1 to 15, following which the surgical wound was closed. The irradiated area showed redness and tenderness but no purulence or drainage. Two weeks following irradiation it was noted that the small treated metastatic nodules began to decrease in size and eventually disappeared. Larger nodules did not undergo marked regressions but showed depression. Maximum decrease in size occurred at 6 to 8 weeks following irradiation. The nodules which had not completely disappeared by then, apparently began to increase again. It would have been of interest to have more information concerning the physical parameters of the nodules under study. Microscopic examination of some nodules which were presumably irradiated revealed phagocytes containing large amounts of pigment rather than viable tumor cells eight weeks post-irradiation

A suggestion that irradiation with lower laser doses over longer periods applied to larger areas resulted in more marked tumor necrosis at an earlier post treatment time does not appear to be substantiated since irradiation data is lacking. Although the authors conclude that the effects could not have been due to thermal factors per se, alternative mechanisms for affecting the course of the tumors were not discussed.

The authors (18) refer to studies on adenocarcinoma which was not affected by laser radiation, unless dyes were introduced into the irradiation site. Even the dyed specimens were found to be less altered by laser radiation than malignant melanoma. The type of adenocarcinoma and a description of the effects would be of interest.

The authors further refer to studies in progress on tissues cultures in order to determine whether a cytotoxic product may be produced. They suggest that an enzyme or a new compound might be formed and indicate that their studies suggest that such a material exists. Although such an observation would be of very considerable significance, no details are presented that would indicate the nature of the preliminary indications for its existence. The authors conclude that no significant therapeutic effect has "yet been demonstrated although some possibilities are suggested" for the management of malignant disease. It is not clear from their report what specific therapeutic possibilities were suggested.

Rosenoff et al (28) described effects following laser irradiation of carcinoma of the larynx and carcinoma of the lung.

Laser radiation was obtained at 6943 Å, at energy levels of 1-3 joules per pulse at a pulse duration of 5 milliseconds. The target spot size on focussing was estimated as 1 mm^2. The laryngeal tumor which had extended to the skin of the left submandillary area presented an exposed area of 4 X 10 centimeters. The beam was focussed with a quartz lens with a focal length of 9 centimeters. Five presumably superimposed exposures, probably focussed to a depth of 5, 6 and 8 centimeters through the center of the lesion were delivered. The half value depth for the radiation is, of course, much less than this. The patient reported no subjective sensations during the irradiation. No reaction at the tumor surface was detected following irradiation. It would be of interest to know how the patients eyes were protected.

Partial necrosis of the tumor was noted over a 6 week period following irradiation. The necrotic area was debrided and a second course of exposures was carried out. One side of the lesion was painted with Evans blue. Irradiation was carried out in a "checker-board distribution" at 1 centimeter intervals. The exposures were carried out on each side focussed to an estimated depth of 1, 2 and 3 centimeters, respectively. Apparently no gross reaction was noted.

Other characteristics of the necrosis were ascribable that would differentiate this lesion from the evident spontaneous areas of necrosis.

Since no indications of burn pathology were observed, the authors, therefore, consider purely thermal effects as unlikely to be responsible for the changes observed, (they do not refer to the considerable literature dealing with this question). They further exclude analogy

to X-ray by the statement that with the power output utilized in the present study, laser is not ionizing, but present no data to support this view. The mechanism of action is considered as possibly similar to that associated with X-rays in that the integrity of the cell membrane system may be disrupted. The patient died 3 weeks following the second course of laser radiation. On microscopic examination of post-mortem specimens, foci of necrosis were found throughout the tumor. Within these necrotic foci, however, rounded areas spaced 1 centimeter apart were noted and were considered as being associated with the second course of laser radiation. The other necrotic areas which were irregular in distribution were assumed to be due to spontaneous necrosis. The authors stated that there were no characteristics by which one form of necrosis could be distinguished from the other. The irradiated sites, however, were sharply circumscribed and showed almost total cellular destruction and basophilic staining of the residual debris. Apparent viable tumor cells were immediately adjacent to the sharply circumscribed necrotic areas related to laser radiation.

Analogous studies were carried out on an apparently subcutaneous lesion which had arisen in a metastasis of carcinoma of the lung. The irradiated mass appeared to have decreased in size within one week of laser irradiation. Further observations during the eight week period which preceded the death of this patient, would have been of interest. Histological studies were carried out on the post-

mortem specimen which indicated random, irregular areas of necrosis into in addition to a "band of necrosis extending the depths of the tumor". The sharply circumscribed borders of this band were interpreted as indicating this area to be representative of the "laser path". The authors further suggest that the basic effect may be a photolytic effect on enzyme systems due to absorption of energy at the proper wave length by enzymes or co-enzymes, particularly those containing heavy metals, as well as a number of other possible mechanisms which are not too well defined. The authors conclude that the volume of tissue destroyed by beams at the energies used by them is limited. In the summary, the statement is made that while lasers destroy human carcinoma in vivo, it is apparently well tolerated by surrounding normal tissue. No evidence is presented concerning irradiation of normal tissue to indicate that it indeed is well tolerated by normal structures. This would have been important since damage to normal tissues due to laser irradiation has been reported by those who have studied effects on normal tissues.

Goldman et al (17) reported observations on 8 patients with various malignant diseases. Two patients with basal cell carcinoma were found to be free of tumor cells for a 3 month period of observation. The statement is made that one lesion which had recurred was treated again with "high energy laser" and cleared without further elucidation.

The third patient had mycosis fungoides in the tumor phase. Three lesions were exposed to laser radiation. No significant changes were observed (on biopsy studies one month following irradiation) at

the sites involved by tumor as compared to control sites which had not been irradiated.

In the fourth patient a superficial melanoma was exposed to laser radiation. Biopsies over an 8 month period failed to show tumor cells except for an area which had received a lower energy density. At that site an intra-epidermal recurrence of melanoma was observed. Six additional exposures at 70 joules per cm^2 were followed by clearing of this lesion.

In the fifth patient, lesions of Kaposi's hemorrhagic sarcoma were irradiated. One of the lesions which was biopsied was in the angiomatous phase. Response to previous treatment with X-ray therapy had resulted in partial regression, which was temporary. Laser radiation resulted in "deep charring and subsequent fading of the target area". Peripheral zones of the residual tumors were present after the nodular portion, which presumably was in the center of the irradiation field, had disappeared. If the irradiation field covered the entire nodule, the lesions appeared to have undergone complete resolution. Lesions which were more extensive, such as erythematous plaques, showed fading which was limited to the irradiation field. Nodular lesions and plaques which had not received X-ray or laser radiation continued to spread. It is not clear from the description whether the partial effect on erythematous plaques was temporary or lasted for the 8 months of the observation period. Lesions which had disappeared entirely, did not recur during this observation period.

It is not clear from the description whether the evaluation of the effects are based on gross inspection as well as microscopic examination. It appears that microscopic studies were carried out,

since the statement regarding activity in the peripheral zone of lesions (not completely covered by the radiation field) would probably have been made on the basis of histological examination. It, furthermore, would have been difficult to come to the conclusion that the tumors had cleared completely and that recurrences were not present in those lesions which had disappeared, unless biopsies were carried out.

The sixth patient was a white female with a 5 year history of Kaposi's hemorrhagic sarcoma. Apparently the patient had responded to X-radiation in the past, but this modality was not used again. Laser radiation was given to recurring nodules and to a nodule which had arisen de novo. Initial charring and crusting which was followed by flattening and the presence of a brown discoloration followed laser irradiation. However, a biopsy obtained 4 months later of one of the nodules which had been irradiated, continued to show the angiomatous phase of the disease. The continued presence of tumor is described as involving scattered areas in which fibrosis had taken place. It is difficult to evaluate the significance of these observations, since lesions which had not received treatment during the period of observation did not appear to have undergone changes. In view of the considerable variability of the course of individual lesions in the same patient, it is difficult to determine what the effects of laser radiation had been. Since apparently, a pretreatment biopsy of the lesion had not been obtained, direct comparison with the post irradiation status is not possible.

Case #7 was a patient who had advanced ulcerative carcinoma of the breast. A heavy fibrinous exudate exposed to laser radiation

showed no gross evidence of changes. After debridement, however, laser radiation produced deep charred areas in the tumor. These studies could not be continued due to death of the patient within two weeks of laser irradiation.

Apparently a biopsy was not obtained from the site of irradiation in this patient. The nature of the changes would have been of interest, in order to determine whether the effects of laser radiation on carcinoma of the breast differed from those produced in the other types of tumors presented in this report.

In the eighth patient an intra-epidermal squamous cell carcinoma (Bowen's Disease) located on the forehead was irradiated. Four days following exposure to laser radiation the entire lesion was excised. Although the patient had been protected with heavy glasses and a black cloth covering his eyes, he reported a bright flash of light. A solar cell which had been placed over his eyelids did not indicate penetration of laser radiation. The possibility that the patient's observation of a bright flash of light was due to transillumination of the tissues was raised. Ophthalmic studies would be of interest.

The authors further comment on the fact that no recurrence has been noted in this lesion. Provided excision was adequate, a recurrence would not be expected, whether or not laser radiation had been employed. Presumably the lack of recurrence is emphasized in order to indicate that laser irradiation of this type of lesion, which is usually confined to the superficial epithelial layers, had not resulted in dissemination or extension of the malignant cells to the deeper layers.

A six month observation period, however, would not be sufficient to ex- this possibility since deeply buried tumor can proliferate for more protracted periods than six months without becoming grossly obvious on clinical examination. The observations made on serial biopsies to exclude the possibility of submerged remnances of the tumor, would be of interest.

The authors indicate that they have carried out studies on lasers capable of an output of 1500 joules in animal tumors. They further indicate that higher levels of energy and larger diameters of the cross section of the radiation are required for the treatment of malignant disease, than those which were employed in the studies recorded. It is not clear from their statement why they did not use concave lenses, since they express their awareness that such lenses would permit an increase of the radiation field. The reduction in energy density would be compensated for by the higher energy output of the unit with an output of 1500 joules.

The authors refer to their studies with glass rods capable of transmitting energy densities exceeding 100 joules, in order to reach otherwise inaccessible lesions. They also refer to studies with gold mirrors of high reflective quality, presumably for the same purpose. A description of the results obtained on tumors with these systems would be of interest.

The authors refer to control studies which indicate that the effects of laser radiation are not due to simple thermal factors.

They refer to studies using cryotherapy, X-ray and grenz ray radiation as the controls. It is unlikely that the levels of energy and power density attained with X-rays or with Grenz rays, were of the same order as those provided by laser radiation. It is difficult, furthermore, to compare cryotherapy to laser radiation. It would furthermore be necessary to give consideration to the rate at which the energy was delivered. Other parameters of the quantitative aspect or qualitative characteristics of the various forms of physical agents employed would have to be taken into account to permit adequate comparison.

The authors further refer to chromosomal changes in tissues cultures as evidence that the cellular changes induced by laser radiation are more than thermal reactions. Presumably this is in reference to the studies of Rounds (28).

The authors emphasize that the data and the studies are preliminary and that the period of observations are too short, the numbers are too few and the studies have been too limited to permit long term evaluation. They suggest "that it is necessary now to explore in a critical investigative fashion" the effects of laser radiation on tumors. They endorse the suggestion made by Ketchum and Minton (4) to institute a cooperative study group comsisting of scientists drawn from the several disciplines involved, as previously suggested by Fine, Klein and Scott for studies on biological effects of laser irradiation (3).

Conclusions

It is evident from the attempt at presenting a critical review of the studies reported on the effects of laser irradiation in man, that these are included primarily to indicate the highly preliminary stages of these studies. In summary, meaningful studies in man must be based on a better understanding of the mechanisms of the interactions of laser radiation with biological systems in general. In view of the rapidly expanding technology of laser devices it is difficult for the studies on the biological effects to keep pace with the rate of technological advance. It is important to recognize, however, that such observations as have been made in man, are not sufficient to provide specific indications of a fundamental difference in the interactions of laser radiation of any type with the tissues of man as compared to those of other mammals. It is further important to recognize for the purposes of this report, that data on the possible hazards to man arising from exposure to laser radiation are insufficient to permit adequate evaluation. Such observations as have been made in man do not exclude the possible hazards that are indicated by studies on small animals (24). Obviously every effort was made in clinical studies to avoid serious effects, whereas in animal studies the opposite was the objective. The studies in man were primarily oriented toward the possible therapeutic applications of laser radiation. The studies in animals, while they included attention to the possible therapeutic aspects of

laser radiation, were directed toward assessing the possible hazards which might arise. However, the rapidly increasing scope and volume of animal and biological in vitro studies on the effects of laser radiation may provide the basis for delineating specific medical indications for the application of laser radiation and for assessing the hazards. At that point a more meaningful analysis of effects which may be specific for human tissues might be made.

1. Litwin, Capt. Martin S., and Glew, Lt. Col. Donald H., The Biological Effects of Laser Radiation, J. Am. Med. Assoc. 187:824-827, 1964.

2. Walt, Ronald A., and Tommes, Charles H., Optical Masers in Biology and Medicine, N. Eng. J. Med. 269: 1417-1421, 1963.

3. Fine, S., Klein, E., Scott, R., Laser Irradiation of Biological Systems, I.E.E.E. Spectrum 1: 61, 1964.

4. Metcham, A., Minton, J., Laser Radiation as a Clinical Tool in Cancer Therapy, Fed. Proc. 24 (1, pt. 3), suppl. 14: S159-163, 1965.

5. Fine, S., and E. Klein, Biological Effects of Laser Radiation, Advances Biol. Med. Phys., 1965 (in press).

6. Goldman, L., Blaney, D., Kindel, D., Franke, E., Effect of the Laser Beam on the Skin. Preliminary Report, J. Invest. Dermatol. 40: 121-122, 1963.

7. Goldman, L., Blaney, D.J., Kindel, D.J., Jr., Richfield, D.F., Owens, F., Human, E.L., Effect of the Laser Beam on the Skin III. Exposure of Cytological Preparations, J. Invest. Dermatol. 42: 247-251, 1963.

8. Goldman, L., Comparison of the Biomedical Effects of the Exposure of Human Tissues to Low and High Energy Lasers, Ann. N.Y. Acad. Sci. 122: 802-831, 1965.

9. Goldman, L. and Wilson, R.G., Treatment of Basal Cell Epithelioma Laser Radiation, J. Am. Med. Assoc. 189:773-775, 1964.

10. Goldman, L., Comparison of Biological Effects of Low and High Energy Lasers, Proc. Boston Laser Conf., 2nd, Boston, Mass, Aug. '63.

11. McGuff, P., Deterling, R., Gottlieb, L., Lamiush Pahimi, Dushnell, D., Roeber, F., The Laser Treatment of Experimental Malignant Tumors, Abstr., J. Am. Med. Assoc. 190 (13): 159, 1964.

12. Fine, S., Hadman, T., Klein, E., Scott, R., Biological Effects of High Peak Power Radiation, Life Science 3: 209, 1964.

13. Goldman, L., Blaney, D., Kindel, D., Richfield, D., Franke, E., Pathology of the Effect of the Laser Beam on the Skin, Nature 197: 912-914, 1963.

14. Klein, E., Fine, S., Hacr, H., Simpson, L., Ambrus, J., Richter, M., Smith, C.W., Mronson, C., Interaction of Laser Radiation with Biological Systems. II. Experimental Tumors, Fed. Proc. 24 (1, pt. 3) suppl. 14: S-143, 1965.

15. Fine, S., Klein, E., Scott, R.E., Seed, R., Laser Radiation in the Syrain Hamster, Skin 2:43, 1963.

16. Fine, S., Klein, E., Nowak, W., Scott, R.E., Loor, Y., Simpson, L, Crissey, J., Donoghue, J., Derr, V., Interaction of Laser Radiation with Biological Systems. I. Studies on Interaction with Tissues, Fed. Proc. 24 (1), pt. 3, suppl. 14:s35, 1965.

17. Goldman, L., Wilson, R., Hornby, P., Meyer, R., Laser Radiation of Malignancy in Man, Cancer, 18:533-45, 1965.

18. Helsper, J.T., Sharp, G.S., Williams, Herbert F., Fister, H.W., The Biological Effect of Laser Energy on Human Melanoma, Cancer, 17:1299-1304, 1964.

19. Goldman, L., Igelman, J.M., Richfield, D.F., Impact of the Laser on Nevi and Melanomas, Arch. Dermatol. 90:71-75, 1964.

20. Klein, E. and Fine, S., The Biological Aspects of Laser Radiation, Abstr. 149th Meeting Am. Chem. Soc., Detroit, Mich., April 1965, p. 41.

21. Rosenoff, H., Hellstrom, R., Brown, J., Carroll, F., Effect of Laser on Carcinoma in Man, J. Am. Med. Assoc. 192:167-168, 1965.

22. Rounds, D.E., Olson, R.S. and Johnson, R.M., The Application of the Laser to Cytology, Proc. Ann. Biomed. Laser Conf., 1st, Boston, Mass., June 1965.

23. Minton, J. and Ketcham, Experimental Results from Exposure of Cloudman S-91 Melanoma in the CDBA/2F$_1$ Hybrid Mouse to Neodymium or Ruby Laser Radiation, presented at Conf. Laser, N.Y. Acad. Sci., N.Y. May 5, 1964.

24. Fine, S., Klein, E., Fine, B.S., Litwin, M., Nowak, W., Hansen, W.P., Caron, J. and Forman, J.: Mechanisms and Control of Laser Hazards and Management of Accidents, 2nd National Conf. on Lasers, Chicago, 1965.

25. Kapany, N.S., Fibre Optics and the Laser, Ann. N.Y. Acad. Sci, 122:615-637, May 1965.

26. Goldman, J., Hornby, P., Long, C., Effects of the Laser on the Skin III. Transmission of Laser Beams Through Fiber Optics, J. Invest. Dermat. 42:231-234, March 1964.

27. Rosenoff, H.L., Hellstrom, R., Brown, J., Carroll, F., Effects of Laser on Carcinoma in Man, J. Am. Med. Assoc. 192:175-176, April 1965.

28. Rounds, D.E., Chamberlain, E.C., and Okigaki, T., Laser Radiation of Tissue Culture, Conf. Lasers, N.Y. Acad. Sci., 1964.

The Purposes of a Study of the Interaction of Laser Beams with the Eye (1)

A. Military:

1. Ocular protection from:
   a. Accidental injury - laboratory, field
   b. Deliberate injury - field
2. Development of weapons to produce:
   a. Temporary blindness (i.e., dazzle or glare)
   b. Permanent blindness (i.e., burn or tissue destruction--scar)
      1. Immediate
      2. Delayed (also possibility that repeated dazzle might lead to delayed permanent effects - thresholds of such possibilities not yet evaluated).
         Delayed - (i) direct or indirect retinal damage
         (ii) to other ocular media such as the cornea and lens..

B. Clinical:

1. Development of a useful clinical tool in ophthalmology (especially in regard to those aspects where the laser may improve on existing photocoagulators such as the xenon arc instrument made by the Carl Zeiss Co.).

2. Ocular protection from accidental injury. Needs differ here from Section A. 1. because of the great difference in power. Clinical instruments are of low power (a few joules output at most). Military instruments are of high power (hundreds to even thousands of joules).
3. Functional studies using attenuated laser beams. Very useful because of their extremely narrow band wave lengths. Such studies are not only necessary for basis understanding of retinal function but are necessary for section A. 2. a.

From the point of view of the eye, there is little question that damage can be produced if sufficient energy reaches the retina and its pigment epithelium (2). Because of direct and indirect (e.g., reflections) exposures which may lie far below those obvious levels of damage, knowledge of a "threshold" for damage to the human eye has been sought (3-6).

The exposure necessary for threshold injury varies in accord with the meaning of the word injury (i.e., temporary or permanent, immediate or dalayed), and in accord with the method used to detect this "minimum injury."

Studies that involve detection of necessary intensities to produce temporary blindness in both light-adapted and dark-adapted individuals are being carried out (6). Such studies have been facilitated by the availability of a reliable, controlled light

source capable of producing a suitable range of intensities, the Meyer-Schwickerath xenon lamp device manufactured by Carl Zeiss Co. in Germany, or a modification thereof.

Availability of reliable laser sources of various intensities and wave lengths, suitably attenuated, might also be used along similar lines. Approaching the problem of "minimum irreversible damage" from another direction, Sperling (7) is investigating flash blindness as a function of wave length specificity on the human fovea. At narrow band wave lengths (2-10 m u) he is able to obtain sufficient intensities for spectral light adaptation, whereas previous workers required wider spectral bands (15-50 m u) to obtain satisfactory adaptation. He found that where very narrow adapting bands in the upper range of intensities of normal vision are used, extreme changes in the shape of the function result. This suggested that after an intense flash sensitivity might be preserved in some parts of the spectrum permitting continuous viewing through special narrow band eye protective filters.

Studies were made using adaptation to white light and narrow band green, yellow and red lights. Further studies are planned to extend these findings to other wave lengths and varying durations of adapting stimuli in an attempt to cover the range up to those intensities that produce irreversible retinal damage.

Flash blindness studies are currently under investigation in a number of laboratories, using white light, attenuated lasers, and modified groupings of lasers ("stacked blinking system".) (6).

In these studies as in those for permanent injuries, "minimum" standards are of importance, exposure vs. recovery time and exposure necessary to initiate irreversible damage.

It is this crucial area of "minimum irreversible damage" that is of greatest importance.

Various methods have been used to determine this level of minimum detectable damage. Ophthalmoscopy was the earliest and most obvious method, but this method varies with the competence of the observer and with wave length of light used to make his observations (generally a red free light source for ophthalmoscopic observation may give lower threshold, even for the less experienced observer).

Histologic methods have been used, including conventional histology, enzyme histochemistry by Geeraets et al. (8), electroretinography by Tengroth et al. and McNeer et al. (9,10) and electron microscopy by B. S. Fine and W. Geeraets (11) in an attempt to 1) pinpoint the site of initial tissue response to a currently acceptable "minimal" exposure; and 2) to determine if a lower threshold can be detected by these or other means, or a combination thereof.

Histologic studies have been carried out to determine clinical thresholds for laser and xenon arc photocoagulation, but the parameters of retinal irradiance and exposure time could not be well controlled in the instruments used (12, 13). (These instruments were primarily designed for clinical use.) Some qualitative differences were, however, observed in that pigmentation appeared earlier (by both ophthalmoscopic and histologic examination) in

the laser-produced lesion than in the lesion produced by xenon lamp coagulation. Such observations are of questionable significance because of lack of control of exposure time and energies used. Similar criticism may be advanced against conclusions which state that the laser lesions occur at a relatively more external level of the retina, as well as other supposed differences, such as subretinal hemorrhages, etc. Conclusions to the effect that the final scar produced by the two instruments is similar appears quite reasonable in view of the fact that tissue reaction in this region is relatively nonspecific.

Other investifations by Ham and Geeraets using instruments (laser and xenon) in which both exposure time and retinal dosage could be more accurately controlled (14), calculated and varied, indicate that under similar conditions, similar changes are produced (3).

These investigators compared the lesion produced by a 175 microsec. exposure from a light coagulation with the lesion produced by a 200 microsec, exposure from a pulsed (non Q switched) ruby laser and concluded there was no difference in effect if time and energy were equal. The retinal doses required to produce a minimal lesion were calculated to be 0.67 joules/cm^2 for light coagulation and 0.72 joules/cm^2 for a pulsed (Non Q switched) ruby laser. Preliminary studies were carried out using a Q switched ruby laser with an exposure time of 27.5 nanosec. The minimum dosage required

to produce a detectable lesion was found to be 0.07 joules/cm^2 The lesions produced by this high power density (2.3 Mw/cm.^{2}) were considered to differ from those produced by a non Q switched laser. They felt that the light coagulation and non Q switched laser lesions could be explained on the basis of calculated temperature rise and thermal conduction, but that q switched lesions in the nanosecond exposure range could not be easily explained by these mechanisms.

Experiments were also carried out to determine the sites within the eye which were responsible for energy absorption (15). In this study by Geeraets and Ham et al. absorption of light energy by 3 pigmented structures, the retinal pigment epithelium, the randomly distributed pigment cells in the choroid and the intravascular pigments were studied. They found that pigment granule distribution varied in the cells of the retinal pigment epithelium but an even greater variation in pigmentation was present in the choroid, an observation in full agreement with existing anatomic knowledge. They noted, by using the albino rabbit, that the blood pigments could only account for 10 per cent of the light incident on the cornea. They concluded that the most important layer for absorption was that of the retinal pigment epithelium, and that in mild lesions this layer may even play the entire role in production of such a lesion. These latter conclusions are also in agreement with previous work by Verhoeff (2) and more recent investigations by B. S. Fine and W. Geeraets (11).

This great importance of the pigment epithelial layer in mild lesons, was considered to be due to the concentration of such a highly absorbing layer in a thin film (approximately 10μ in man) as contrasted with a layer of similar absorbing ability (the choroid) wherein the absorbing cells are distributed throughout a layer 100 to 200 μ thick (in man).

This calculation based on conventional anatomic studies, is strengthened by more recent electron microscopic studies (4,11) that narrow the pigment layer to as little as 1/3, or no more than 1/2 of the thickness of the pigment epithelial cell.

More recently Pomerantzeff (5) pointed out that this variability of pigmentation in both pigment epithelium and choroid would result in greater variability in absorption for a (clinical) laser output than for a (clinical) xenon lamp discharge.

Such biologic variability would handicap determination of an accurate or pinpoint minimum detectable biologic response. A range of exposure for a variety of minimal detectable changes would therefore be more likely. He also noted (5) that if a very light pigment epithelium were to be superimposed on a very darkly pigmented choroid, the penetration of the laser beam would be much greater than anticipated for "average" calculations.

Previous experiments by Geeraets et al (16) indicated that for exposure times greater than 0.3 seconds heat loss due to blood flow (choroid)

and tissue conduction increase substantially the amount of energy required to produce a threshold chorioretinal lesion.

Later work indicated that for short exposure times in the millisecond range, mild retinal lesions were similarly produced by both laser and white light sources and could be adequate explained on the basis of thermal damage in the pigment epithelium and in the retinal regions immediately adjacent to the pigment epithelium.

They felt that lesions produced by higher power densities and shorter exposure times (Q-switched pulses in the nanosecond range)(3) could no longer be explained on the basis of thermal injury. Further investigation of these short duration high density exposures is therefore necessary.

Many of these observations for pulsed white light and pulsed (non Q-switched) ruby laser radiation were supported by more recent light and electron microscopic observations (11). This latter study indicated that the primary site of reaction to a mild or minimal insult lay in the retinal pigment epithelial cell, more specifically, in the system of smooth-surfaced endoplasmic reticulum which surrounds the pigment (melanin) granules. The thickness of the melanin granule layer of the pigment epithelium was noted to be even thinner (and therefore even more highly concentrated in a single plane) than previously estimated from the conventional anatomic literature.

Evaluation of the shorter duration higher power density (Q-switched ruby laser) threshold lesion is planned.

Threshold lesions demonstrable by conventional histologic techniques were found to be ophthalmoscopically detectable (8). Therefore, attempts

were made to detect morphologic changes below these histologic methods by demonstrating enzyme inactivation by histochemical studies, using precipitated nitroblue tetrazolium to form insoluble formazan salts from reintal diphosphopyridine nucleotide diaphorase activity (DPNH) (an oxidative enzyme function present in mitochondria) before and after insult. These enzymes are therefore encountered in high concentrations in the ganglion cell and outer plexiform retinal layers, sites where mitochondria abound. They are also present in the pigment epithelium layer, but the insoluble salts produced by the tetrazolium reaction are easily obscurred by the pigment granules. These investigators found that by such enzymatic inactivation studies, there was minimal spread of thermal conduction (and so damage) at short exposure times (175 microsec.) but with longer exposure times (30 microsec.) there was significant "spread" of inactivation (and so damage) from the edge of the irradiated zone, depending on the intensity of the energy delivered. They were able to detect changes in the sensory retina (i.e., photoreceptor ellipsoids) by such impairment of enzymatic activity when the energy of the exposure light beam had been reduced to 10 to 15 per cent below threshold for an ophthalmoscopically-visible or histologically detectable lesion. Changes in the pigment epithelium were apparently not satisfactorily detected by these methods.

All of these observations and experiments it must be noted, have been carried out mainly on the rabbit eye, using a pulsed non Q-switched ruby laser. Preliminary observations by Geeraets and Ham indicate that use of a Q-switched ruby laser lowers the retinal threshold of damage to a tenth (3) i.e. 0.07 joules/cm^2. Other varieties of laser, both pulsed and continuous at the various wave lengths becoming available

may have differing "threshold" values for retinal injury.

The electroretinogram (ERG) has been used by Tengroth et al. and McNeer et al. in an attempt to detect lower thresholds of damage.(9,10). Unfortunately, current interpretation of the E R G is controversial (10, 49), and its value for such a study may be severely limited as has been pointed out (41). They caution that objective reduction in B wave amplitude after light exposure may not necessarily equate with functional disturbance, a relationship difficult to determine in a rabbit.

McNeer et al. (10) have shown that B wave changes can be demonstrated after light exposure, with energies 50 per cent below that required for ophthalmoscopically visible lesions if a sufficient area of the retina were involved. This area of retinal exposure was found to be approximately 40 m.m. (2) They used a delay period of three days before ERG evaluation to avoid various artifacts. A single lesion of 0.75 mm. diameter (detectable by conventional light microscopic methods) (3) was insufficient to obtain an ERG change and therefore was not detectable by this method. These authors point out the work of others (Aronson and Garoutte) (50) who showed that the amplitude of the B wave could be reduced in linear fashion with visible retinal burns when 12 to 49 per cent of the working retina was involved. Other complicating factors pointed out were those of the normal day-to-day variation in ERG amplitudes (51).

Tengroth et al. (9) used a human volunteer prior to enucleation of an eye for carcinoma of the orbit and tested the ERG with both ruby laser flashes and white light photostimulation. With laser stimulation

alone there was absence of the A wave and the B wave was of slightly greater amplitude but shorter duration than those present after normal white light stimulation. No change could be detected using the usual white light photostimulation to evoke the ERG after a laser impact. They felt that the slight laser flash induced ERG changes were unlikely to be a local response from the retina because of the small area (i.e. approximately 0.07 mm^2) of the retina exposed. They concluded that this slight response was a photopic effect from the whole retina due to scattering of the light.

Tengroth et al (9) also noted that in their human subjects no real dazzling was obtained with the flash from a ruby rod laser, but exposure to a Karpe photostimulator did produce a dazzling effect. This effect they thought might be due to the peculiar (red) and narrow band (6943Å) wavelength which might fail to stimulate a large number of relatively red insensitive retinal rods.

These observations point up a potential serious flaw in all the experimental models using the rabbit retina. Although most investigators felt that data obtained from the rabbit retina may be easily extrapolated to the human, some doubt must exist if this is true for the macula and foveal areas, for the rabbit does not possess this critical area. The rabbit retina, unlike the primate, is also, for all practical purposes nonvascularized. If damage to the human eye is the primary concern then damage to the critical areas of macula and fovea are of primary concern. Small lesions in the peripheral retina are unlikely to cause any functional disturbance (other than possibly temporary dazzling effects discussed previously or intraocular hemorrhages, to be noted later).

Preliminary investigations have been carried out by Jones and McCartney (52) using primate (i.e. monkey) eyes exposed to laser pulses of 5 to 250 joules using a Maxwellian view. By this means they illuminated approximately 24 percent of the total retinal area (78.5 cm^2). At outputs above 5 joules they produced total disruption of the lens and gas bubbles in the anterior chamber. Considerable intraocular damage also resulted but is difficult to evaluate from their published data.

Investigations of similar effects on the rhesus monkey macula and foveal areas are unfortunately accompanied by a number of serious technical difficulties that are not present in the rabbit. For example, only a single fovea and macula is present in each eye so that no great number of experiments can be conducted in any single primate eye. These problems will make "threshold" damage detection in the primate macula and fovea extremely difficult.

Although there are many points of anatomic interest in the region of the fovea (53), such as a consideration of the structure of the rodlike cones, and the possible difference in height of the pigment epithelium, the ease of formation of artifactitious "edema" or swelling in these retinal layers, and detachment of this area makes interpretation of experimental "threshold" lesions very difficult. Generally, only small fragments of foveal photoreceptor outer segments can be obtained for such studies as electron microscopy, although characteristic inner segments and synaptic areas are readily preserved (54). Very few electron microscopic studies have yet been made on this area (55).

Protective devices are basically of two kinds: (1) A device to attenuate the entire exposure to below levels of injury either temporarily or permanent; (2) Development of a narrow band eye protective filter which might permit continuous viewing through some parts of the spectrum

where sensitivity is preserved after an intense flash.μ

Knowledge of a "minimum" threshold of damage therefore becomes of considerable importance.

Straub, using the previous data of Ham and Geeraets based on their non-Q-switched ruby laser studies in the rabbit retina, and taking a safety factor of 100, constructed a protective glass of Schott type BG-18 filter glass suitable for the wavelength of a ruby laser. Unfortunately, with development of Q-switching devices raising the power levels of the laser, as well as proliferation of lasers of various wavelengths, the usefulness of this device becomes severely limited.

Because variation in fundus pigmentation appears to determine susceptibility to retinal damage, a means of estimating this susceptibility *in vivo* by use of light reflectance from the ocular fundus was attempted by Geeraets *et al* (18). They used a light meter bridge and a vacuum phototube (peak sensitivity 400 mμ) attached to a Zeiss fundus camera. A later modification used a photomultiplier tube in lieu of the phototube, in order to be able to measure narrow spectral bands as well as to permit measurements in human eyes where the amount reflected is lower than in the rabbit, and stronger illumination would not be well tolerated by the subject.

Fundus light reflectance was therefore measured arbitrarily in microamperes and this was charted against transmission through the various ocular layers determined by rapid dissection of the eye following exposure, and determination of transmission coefficients using a DK-1 spectrophotometer. They found that exposure time for the production of minimal lesions did not increase linearly with increase in per cent transmission, especially beyond transmissions of 50 per cent. When

exposure time in milliseconds was plotted against light reflectance in arbitrary (microamperes) units they found that useful correlation did exist and was also applicable to the human eye.

Although this technique may be of considerable value in choosing rabbits (or other experimental animals) with approximately uniformly pigmented fundi for experimental purposes, this technique appears, at the moment, to be of limited value in the clinical (either military or civilian) situation.

Search for suitable protective devices is of greatest importance. Accidental permanent injury due to a ruby rod laser flash has already been reported (in the unclassified literature) from at least one laboratory (19).

Damage from exposure to atomic flash is well documented by Byrnes et al and Rose et al (20-22). and this problem is further aggravated by the greater and longer intensity flash of high altitude nuclear explosion.

If current protective devices under development should succeed in preventing permanent ocular damage, they may only attenuate the flash with temporary incapacitation as a persisting hazard. Such a hazard is of great importance to the pilot of high-speed aircraft. Suggestions have been made (6) that if there is failure to protect the pilot in full, the instrument panel might be flooded with white light so that the dazzled pilot might see enough information by extra-foveal vision to protect himself through this temporary period of disability.

In all of these studies it is probable that the macular and foveal (cone or photopic) vision of the pilot is of greatest importance. Peripheral (rod or scotopic) vision may be of much lesser importance,

whether flying is by day or night. It is highly unlikely that damage to peripheral vision alone either by permanent injury or by dazzle will incapacitate the pilot unless perhaps he suffers an intraocular hemorrhage. Focal damage or dazzle to the fovea or macula will incapacitate him greatly.

The ocular media, however, are stated by Pomerantzeff to absorb 25 per cent of the light from a xenon lamp source as compared with 5 per cent of the light from a pulsed ruby rod laser source (5,56) probably due to the higher content of infrared in the xenon source.

Until recently, absorption by the ocular (transmitting) media of wavelengths from approximately 3,000 Å to 8,000 or 9,000 Å at low intensities has been minimal and nondamaging. Wavelengths above and below these limits are increasingly absorbed by the ocular media. This is especially true of the infrared. To reach the retina their energies must be great. Recent developments in laser research (e.g., neodymium doped glass, wavelength 10,600 Å) are making available these wavelengths with very high energy density levels such that immediate and delayed effects on the cornea, lens and even the retina (either primary or secondary) may be anticipated.

Although certain nonlinear effects such as the Raman effect, secondary harmonic generation, acoustic wave generation and damage to transparent media are possible with the power densities of lasers of great output, only acoustic wave generation seems likely to be produced by a clinical laser coagulator (40). Such acoustic effects may therefore result from the lower intensities that may be expected from accidental reflections from the target on irradiation with high intensity sources.

Clinically, lasers are ruby rod devices of small output (12,25, 27,34a) and retinal vessels were relatively unharmed, presumably due to their reflectance of the red light. Higher energy laser sources may increase the hazard of intraocular hemorrhage especially those devices generating wavelengths easily absorbed by the blood in the retinal vessels. With this method long-term incapacity may be produced even by irradiance (accidental or deliberate) of the peripheral retina.

In threshold studies with a ruby laser, Campbell et al have obtained preliminary results that indicate the energy for a threshold lesion obtained with a laser beam are consistent with Ham's Xenon arc data (59).

Some consideration must be given in threshold determinations to various factors. The non-uniformity of the irradiating beam will result in "hot spots." The resultant energy density distribution throughout the beam will not be constant. The power density distribution throughout the beam will also vary as a function of time. The output pulse from the ruby laser is not continuous, but consists of a series of spikes. The effect, therefore, may differ from that produced by a non-spiking system whose parameters are otherwise similar. The significance of this factor may be dependent on the thermal parameters of the primary absorbing site, which is probably the pigment epithelial cell. Furthermore, the threshold in joules/cm^2 may vary as a function of the retinal area irradiated.

Other studies on the interaction of laser radiation with the eye have been directed towards determining effects at energy levels in excess of those required to produce threshold injury. The first reported studies on biological effects of laser radiation, the production of ocular lesions were initiated by Zaret et al (57). The maximally dilated pupils of adult pigmented rabbits were irradiated using a ruby laser (0.1 joules per pulse 0.5 millisecond pulse duration). On irradiation of a region containing medullated nerve fibers proximal to the optic nerve head, a site where energy absorption is least efficient, the visible lesion was minimal, but bubbles were produced in the vitreous. When directed towards pigmented regions, the lesion consisted of blanched coagulated retina, elevated in crater fashion, and containing a small, centrally placed hemorrhage. Five days later, the appearance of the lesion was that of a flat white scar with pigment clumping in and around the area. Following multiple exposures the hemorrhage extended into the vitreous. Focused irradiation of the pigmented rabbit iris by means of a short-focus lens, resulted in a characteristically dark brown and irregularly shaped lesion. Several days later the pupil constricted in a grossly eccentric fashion. In further studies on eyes of brown and chinchilla grey rabbits, a central zone, which contained a black deposit, together with a chorioretinal hole, occasionally obscured by a small gas bubble or hemorrhage extending into the vitreous was observed (34a).

An annular region of blanched, coagulated retina began to evolve 1-3 seconds after exposure. A region of retinal edema which had the appearance of a halo surrounded the coagulated zone. The lesion in the brown and grey rabbit was similar. Reduction of the beam energy resulted in smaller areas of coagulation. Light microscopic studies were carried out, and were well described. (The resultant cicatricial chorioretinal adhesion was similar to that produced by other light sources).

Initial experimental studies by Koester, Snitzer, Campbell and Rittler were reported, using a 0.04 joule, 0.8 millisecond ruby unit (58). Further studies carried out by Campbell, Rittler and Koester included a large series of studies on eyes of adult pigmented rabbits using a laser and Xenon-arc photocoagulator (12). In these animal studies, the lesions produced by the laser appeared to be at a more external level of the retina but were qualitatively similar to those produced by the Xenon arc unit. On microscopic examination, pigmentary changes appeared to occur earlier after laser irradiation.

The occurrence of vitreous bubbles may consequently be of importance in considering the laser for a clinical tool. Follow-up of the lesion over a period of years is desirable.

Trans-scleral application of laser techniques by Campbell et al (28) has been attempted using a fiber optic bundle of neodymium-doped glass as the laser source. This approach was developed because of clinical difficulties in treating lesions of high reflectance or lesions located in the extreme periphery of the globe where location and optical inhomogeneities limit the transpupillary approach. Although clinical application

of this technique is limited due to technical difficulties of maintaining integrity of the fiber bundle with repeated exposure, these experiments point up the possible hazards of trans-scleral injury from directed or misdirected powerful laser sources. Deep intraocular damage (without transmission through the pupil) is a possibility that is currently under investigation by Neidlinger (29).

With development of high intensity laser the possibility of cataract formation, either immediate or delayed, has been considered by Zaret (56). It was once felt that true cataracts could be produced by direct absorption of infrared by the lens (Vogt,30), but subsequent work of Goldman (20) and Langley et al (30), indicate that the cataracts produced are dependent upon absorption of light by the overlying pigment epithelium of the iris with conversion of the energy to heat. The underlying lens epithelium is damaged by the heat with formation of a focal anterior subcapsular cataract (swelling and even necrosis of the epithelial cells). Subsequent faulty proliferation and posterior subcapsular migration of the injured cells produced a posterior subcapsular cataract of clinical significance, the latent period being of the order of 60 to 90 days. This latent period was considered to be variable depending on the amount, duration and distribution of the irradiating dose. Radiation of the peripheral iris which does not make contact with the lens, or the albino iris which has no pigment, failed to produce lens changes.

Very long term cataractogenesis as in x-radiation is not to be expected from white light photocoagulation, especially those of clinical usefulness. Recent evidence indicates that the higher density output of laser devices cause ionization (31) or free radical formation in tissue not unlike the effects of x- and gamma radiation. If true, delayed cataractogenesis is possible and must be considered. Once again the medicolegal implications may become exceedingly complex because of the natural incidence of small peripheral lens changes in older individuals. These peripheral, generally spoke-like cataractous changes differ slightly in their clinical appearance from the more globular pre-equatorial changes that might be observed following ionizing radiation in the early stages. Later ionization changes may be indistinguishable from senile cataractous change.

Although such damage which may produce an iritis may be of lesser importance in the acute military situation (i.e., pilot, etc) because the pupillary portion of the lens is not involved, these delayed effects are definate hazards in the laboratory and clinical situation where higher powered laser sources are being used experimentally, or in the attempts to produce optical or filtering peripheral iridectomies (26,32-34a). Even iritis, if recurrent can be a hazard in producing a chronic situation which may ground a highly trained pilot.

In the clinical situation use of moderate to intense radiation may provoke an intraocular hemorrhage from the choroid or retraction of the collagenous vitreous framework with the added complications of delayed retinal detachment and the formation of fixed retinal folds. In the experimental and military situation where sources of greater power are being used, these immediate and delayed complications are an even greater potential hazard. Although heating of the vitreous body has been considered to be minimal (35-38) the effect of cummulative heating on the vitreous framework collagen is not

known. Thermal summation in the vitreous body has been considered more likely with xenon arc photocoagulation devices than the pulsed ruby rod because of the greater duration of the irradiation and greater absorption by the ocular media (5). It may be anticipated that this discrepancy will disappear with prolonged exposures to a continuous laser source.

Direct thermocouple measurements as a means of accurate temperature recording in biological structures gives rise to many artifacts of reflectance, absorption and conduction at the very site in the tissue where measurements are being made. The thermocouple is generally large in size in relation to the illuminating beam and its response time too slow for the rapidity of the temperature rise (36,38,41).

Because of the greater attachment of vitreous to retina in the periphery and because of increasing (physiologic) degeneration of the retina (cystoid degeneration) eccentric (peripheral) exposure to repeated low elevations of temperature may be a distinct hazard in regard to accentuating retinal tears and detachment. Heavy coagulation damage to the peripheral retina which does not impair vision has been known in clinical photocoagulation to produce cystoid changes in the distant critical macular area with drop in vision. These possibilities conjure up a nightmare of medicolegal possibilities in retinal detachments and decreased vision occurring in personnel engaged in laser activities.

An earlier, well done study of photocoagulation effects on the dog eye by Okun et al (39)(Zeiss-Meyer-Schwickerath clinical apparatus) indicated that mild lesions might be more damaging in the long run because of a proliferative response of the glial cells which remain viable, together with a condensation effect on the adjacent vitreous to produce vitro-retinal adhesions and potential traction bands.

Other complications such as choroidal rupture, hemorrhage, irido--cyclitis were attributed to excessive photocoagulation.

These observations followed up for as long as eight months after exposure, support a cautious acceptance of the safety conclusions arrived at by such means as temperature measurements made in the vitreous body immediately after a few impacts. Much long-term clinical observation and experience supports the experimental findings of Okun and Collins (39).

Cibis (42) and Zaret (56) have pointed out many of the hazards in clinical photocoagulation such as overheating of the anterior chamber by inadvertently striking the iris, the production of thermal cataracts, vitreous hemorrhages from retinal or choroidal vessels, secondary retinal tears and eventual retinal detachment at the atrophic periphery of old chorioretinal scars, retinal holes due to necrosis of retinal tissue, t transient exudative retinal detachment and macular deterioration by traction from coagulation scars. Cibis also points out that albinotic eyes are not immune to high energy injury and that there is a reduction in viability of ocular tissues with aging.

The retina has long been known to be susceptible to a variety of chemicals, a number of which have been widely used for therapeutic purposes. Chloroquine retinopathy has been known for approximately five or six years (43).

In addition to these antimalarial drugs, others such as the phenothiazine (and other polycyclic compounds) are being widely used in high dosages. Recent experimental work by Potts (44,45,45a) has shown that these latter categories of medications are sequestered into the melanin granules of the choroid and retinal pigment epithelium, the sites where

the light energy is believed to undergo transformation into damaging heat. The potential retinotoxic effect of these drugs, like other drug hazards (46,47), might be potentiated by exposure to excessive photic energy (i.e., the retina under medication might become more susceptible to photic injury -- a lowering of the threshold for damage). A wide variety of therapeutic agents are known to be photosensitizing (47), at least to the skin. The lens of the eye, embryologically a derivative of the surface ectoderm, should be suspect of similar responses.

Exposure to high oxygen pressures (hyperbaric oxygen), widely used for therapy and environmental research, has been shown by Beehler to produce retinal hemorrhage, retinal detachment, hypotony and iritis in dogs in as little as 48 hours (48). Although these changes were in part reversible, they may potentiate retinal susceptibility to photic injury. These possible threshold lowering agents may be of greater importance when one is concerned with a potentially more susceptible human macula or retina.

LASER PHOTOCOAGULATORS: (Clinical devices commercially available)

1. American Optical Company (U.S.)

   - water cooled ruby rod device - floor mounted device.

   - single telescopic viewing; indirect ophthalmoscope with erect image by optical inversion - continuous fundus view during firing.

   Approximate cost $13,000.

2. Optics Technology Incorporated (U.S.)

   - ruby rod device in hand held direct ophthalmoscope

   - special adaptor available for indirect ophthalmoscopic viewing

   - mechanical shutter obstructs view during firing

   - 2 models available with costs ranging approximately $4500 to $6000.

3. Vernon-Ingram Laser Ophthalmoscope by Keeler-Nelas (England)

   - hand held ruby device in direct ophthalmoscope - continuous fundus view during firing.

   Approximate cost $7,000 before customs duty.

4. Maser Optics Incorporated (Boston, Mass. U.S.)

   - floor model ruby device using a built-in indirect binocular ophthalmoscope - continuous viewing during firing.

   Approximate cost $7,000.

5. Experimental Unit - not yet commercially available:

   Minneapolis-Honeywell, hand held direct ophthalmoscope - in trial use at Mayo Clinic, Rochester, Minnesota

   No price.

6. Clinical xenon lamp photocoagulators - commercial

   1. Carl Zeiss (Germany) - Meyer-Schwickerath apparatus - direct ophthalmoscopic view. Approximate cost $13,000

   2/ Zeiss, Jena (Germany - east) - binocular viewing. Approximate cost $13,000

REFERENCES

1. Fine, S., Klein, E.: Biological effects of laser radiation. Advances in Biomedical Physics, Academic Press, in press, 1965.
2. Verhoeff, F.H., Bell, L., Walker, C.G.:The pathological effects of radiant energy on the eye. Proceedings of the American Academy of Arts and Sciences. 51: No. 13, 603, July 1916.
3. Geeraets, W.J., Ham, W.T., Jr., Williams, R.C., Mueller, H.A., Burkhart, J., Dupont Guerry, III, Vos, J.J.: Laser versus light coagulator: a funduscopic and histologic study of chorioretinal injury as a function of exposure time. Federation Proc. Suppl. 14, Vol. 24 #1, Pt. III, S-48, Jan-Feb, 1965.
4. Fine, B.S.: Limiting membranes of the sensory retina and pigment epithelium: an electron microscopic study. Arch. Ophth. 66: 847-860, 1961.
5. Pomerantzeff, O.: A comparison of the physical aspects of xenon-arc and laser photocoagulation. International symposium on new and controversial aspects of retinal detachment. Houston, Texas June 3-5, 1965.
6. RACIC report. State of the art study on visual impairment by high intensity flash of visible, infrared or ultraviolet light. Report No. BAT-171-9 under contract SQ-171 by Christner, C.A., Cress, R.J., Drumheller, R.A. Hassfurther,M.E., McFarland, R.R., Bugbee, N.M. Remote area conflict information center, Battelle Memorial Institute, Columbus, Ohio.

7. Sperling, H.G.: Flash blindness as a function of wavelength specificity. Federation Proceedings 24: Suppl. 14, #1, Pt. III 5-73, Jan-Feb 1965.

8. Geeraets, W.J., Burkhart, J., Guerry, D., III: Enzyme activity in the coagulated retina: A means of studying thermal conduction as a function of exposure time. Acta Ophthalmologica, 76: 79, 1963.

9. Tengroth, B., Karlberg, B., Bergqvist, T., Adelhed, T.: Laser action on the human eye. Acta Ophthalmologica, 41; 595, 1963.

10. McNeer, K., Ghosh, M., Geeraets, W.J., Guerry, D., III.: Electro retinography after light coagulation. Acta Ophthalmologica Suppl. 76: 94, 1963.

11. Fine, B.S., Geeraets, W.J.: Observations on early pathologic effects of photic injury to the rabbit retina: a light and electron microscopic study. Acta Ophthalmologica, in press, 1965.

12. Campbell, C.J., Rittler, M.C., Koester, C.J.: The optical maser as a retinal coagulator: an evaluation. Trans. Am. Acad. Ophthal. 67: 58-67, Jan-Feb, 1963.

13. Noyori, K.S., Campbell, C.J., Rittler, C., Koester, C.J.: The characteristics of experimental laser coagulation of the retina. 72: 254, 1964.

14. Ham, W.T. Jr., Williams, R.C., Ruffin, R.S., Schmidt, F.M., Mueller, H.A., Guerry, D. III., Geeraets, W.J.: Electronically pulsed light source for the production of retinal burns. Acta Ophth. Suppl. 76: 59, 1963. (Abstr. of Med. Electr. article)

15. Geeraets, W.J., Williams, R.C., Chan, G., Ham, W.T. Jr., Guerry, D.III, Schmidt, F.H.: The relative absorption of thermal energy in retina and choroid. Invest. Ophth. 1: 340, 1962.

16. Geeraets, W.J., Williams, R.C., Ham, W.T., Guerry, D. III, Rate of blood flow and its effect on chorioretinal burns. Arch. Ophth. 68: 88, 1962.

17. Ocular effects of laser radiation Part I. Depts. Biophysics and Ophthalmology, Medical College of Virginia. DASA-1574, Contract DA-49-146-XZ-102, Defense Atomic Support Agency, Washington, D.E.: Ham,W.T., Williams, R.C., Mueller, H.A., Ruffin, R.S., Schmidt, F.H., Clarke, A.M. and Geeraets, W.J.

18. Geeraets, W.J., Williams, R.C., Ghosh, M., Ham, W.T. Jr., Guerry, D.III, Schmidt, F., Ruffin,R.,: Light reflectance from the ocular fundus - a means of estimating susceptibility to retinal burns. Arch. Ophth. 69: 612, 1963.

19. Blancard, P., Sorato, M., Blonk, K., Iris, L., Liotet, S.: A propos D'une Photocoagulation maculaire par laser, accidentelle. Ann. O'culist. 198: 263, March 1965.

20. Byrnes, V.A., Brown, D.V.L., Rose, H.W., Cibis, P.A.: Chorioretinal burns produced by an atomic flash. Arch. Ophth. 53: 351, 1955.

21. Byrnes, V.A., Brown, D.V.L., Rose, H.W., Cibis, P.A.: Retinal burns - new hazard of the atomic bomb. J.A.M.A. 157: 21, 1955.

22. Rose, H.W., Brown, D.V.L., Byrnes, V.A., Cibis, P.A.: Human chorioretinal burns from atomic fireballs. Arch. Ophth. 55: 205, 1956.

23. Whiteside, T.C.D.: Dazzle from nuclear weapons - Vision Research Reports, III. Papers presented at 39th Meeting, April 4-5, 1960, 79-95 (AD-252513) quoted in RACIC report, page 32.

24. Culver, J.F., Newton, N.L., Penner, R., Neidlinger, R.W.: Human chorioretinal burns following high altitude nuclear detonations. Aerospace Medicine 35: 1217, 1964.

25. Flocks, M., Zweng, H.C.: Laser coagulation of ocular tissues. Arch. Ophth. 72: 604, 1964.

26. Zweng, H.C., Flocks, M., Kapany, N.A., Silbertrust, N., Peppers, N.A.: Experimental laser photocoagulation. AJO 58: 353, 1964.

27. Havener, W.H.: Technical aspects of laser coagulation. AJO 58: 38, 1964.

28. Campbell, C.J., Noyori, K.S., Rittler, M.C., Innis, R.E., Koester, C.J.: The application of fiber laser techniques to retinal surgery. Arch. Ophth. 72: 850, 1964.

29. Neidlinger, R.W.: Walter Reed General Hospital, Department of Ophthalmology - personal communication

30. Langley, R.K., Mortimer, C.B., McCulloch, C.: The experimental production of cataracts by exposure to heat and light. Arch. Ophth. 63: 473, 1960.

31. Fine, S., Klein, E., Fine, B.S., Litwin, M., Nowak, W., Hansen, W.P., Caron, J., Forman, J.: Mechanisms and control of laser hazards and management of accidents. Proc. 2nd classified laser conference, Chicago 1965.

32. Meyer-Schwickerath, G.: Light coagulation. Tr. S.M. Drance, C.V. Mosby Company - St. Louis, Mo., 1960.

33. McDonald, J.E., Light, A.: Photocoagulation of iris and retina. Arch. Ophth. 60: 384, 1958.

34. Hogan, M.J., Schwartz, A.: Experimental photocoagulation of the iris of guinea pigs. AJO 49: 629, 1960.

36a Zaret, M.M., Ripps, H., Siegel, I.M., Breinin, G.M.: Laser photocoagulation of the eye. Arch. Ophth. 69: 97, 1963.

35. Noyori, K.S., Campbell, C.J., Rittler, M.C., Koester, C.: Ocular thermo effects produced by photocoagulation. Arch. Ophth. 70: 817, 1963.

36. Najac, H., Cooper, B., Jacobson, J.H., Shamos, M., Breitfeller, M.: Direct thermocouple measurements of temperature rise and heat conduction in the rabbit retina. Invest. Ophth. 2: 32, 1963.

37. Campbell, C.J., Noyori, K.S., Rittler, M.C., Koester, C.J.: Intraocular temperature changes produced by laser coagulation. Acta. Ophth. Suppl. 76: 22, 1963.

38. Crowder, J.: Measurements of the vitreous temperature during photocoagulation in the rabbit eye. Acta. Ophth. Suppl. 76, 32, 1963.

39. Okun, E., Collins, E.M.: Histopathology of experimental photocoagulation in the dog eye: Part I, Graded lesions, vitreous effect and complications. AJO 54: 3, 1962.

40. Koester, C.J.: Energy density considerations in laser photocoagulation. International symposium on new and controversial aspects of retinal detachment. Houston, Texas, June 3-5, 1965.

41. Geeraets, W.J., Ridgeway, D.: Retinal damage from high intensity light, Acta Ophth. Suppl. 76: 109-112, 1963.

42. Cibis, P.A.: Limits and hazards of photocoagulation. Symposium: Photocoagulation, Trans. Am. Acad. Ophth. & Otol. 66: 71-87, 1962.

43. Bernstein, H.N., Ginsberg, J.: Pathology of chloroquine retinopathy. Arch. Ophth. 71: 223, 1964.

44. Potts, A.M.: The concentration of phenothizanes in the eye of experimental animals. Invest. Ophth. 1: 522-530, 1962.

45. Potts, A.M.: Further studies concerning the accumulation of polyclinic compounds on uveal melanin. Invest. Ophth. 3: 399-404, 1964.

45a Potts, A.M.: The reaction of uveal pigment in vitro with polyclinic compounds. Invest. Ophth. 3: 405-416, 1964.

46. Cutting, W.C.: Guide to drug hazards in aviation medicine. Fed. Aviation Agency, Aviation Medical Service, 1962.

47. Young, J.W.: A list of photosensitizing agents of interest to the dermatologist, Bull. Assoc. Military Dermatologists. 13: 33, March 1964.

48. Beehler, C.C., Newton, N.L., Culver, J.F., Tredici, T.J.: Retinal detachment in adult dogs resulting from oxygen toxicity. Arch. Ophth. 71: 665, 1964.

49. Jacobson, J.H., Najac, H.T., Stephens, G., Kara, G.B., Gideon, F.G.: The role of the macula in the electroretinogram of monkey and man. AJO 50: 5, Pt. II, Nov 1960.

50. Aronson, S.B., Garoutte, W.: The effects of retinal lesions on the ERG. Invest. Ophth. I: 3, 416, June 1962.

51. Spivey, B.E., Pearlman, J.T.: Day to day variations in the ERG of humans and rabbits. Invest. Ophth. I: 3, 432, June 1962.

52. Jones, A.E., McCartney, A.J.: The effect of high energy ruby laser pulses on primate ocular structures. Abstr. - Biological sessions - Boston laser conference, Northeastern University, Boston, Mass. Aug 5-7, 1964.

53. Polyak, S.L.: The retina. Univ. of Chicago Press, Chicago, Ill, 1948.

54. Fine, B.S.: personal observation

55. Dowling, J.E.: Foveal receptors of the monkey retina: Fine structure. Science 147: 57-59, 1965.

56. Zaret, M.M., Fed. Proc., 24(1) Pt III, Suppl 14,:S-62, 1965.

57. Zaret, M., Breinin, G.M., Schmidt, H., Ripps, H., Siegel, I.M., and Solon, L.R., Science, 134, :1525, 1961.

58. Koester, C.J., Snitzer, E., Campbell, C.J., and Rittler, M.C., J. Opt. Soc. Amer., 52:607, 1962.

59. Swope, C.H., and Koester, C.J., paper presented at Opt. Soc. Am. Spring Meeting, Washington, D.C., 1964.

Dentistry

Interaction of laser radiation with oral hard tissues was studied by Lobene et al (1). Extracted human teeth were exposed to laser radiation at a wave length of 6934Å at energy levels of 12-25 joules. Unfocused, defocused and focused beams were used. A lens system provided spot sizes less than 1mm. in diameter at the point of impact, resulting in estimated power densities exceeding 1 Mw/cm^2. Duration of impact was of the order of 1 millisecond per exposure. The penetration of intact enamel was approximately 1mm. An area of enamel with a fused glass-like appearance 4-8 mm. in diameter surrounded each opening. Root comentum and adjacent dentine were penetrated with charring of the surface cementum. Undecalcified sections revealed an amorphous zone of enamel immediately adjacent to the cavity and disruption of the rod structure from the apex of the cavity to the D.E. junction. Changes were not observed in the adjacent dentine. When examined under polarized light, reduced birefringence was seen in the amorphous zone and the disrupted enamel rods adjacent to the cavity. Enamel exposed to defocused laser radiation at an energy density less than 100 joules/cm^2 showed no gross surface changes. Sections examined showed disruption of enamel rods similar to that observed in the cavity area produced at higher energy densities.

Xray diffraction patterns, microhardness, and solubility of irradiated enamel were also studied. The specimens were examined microradiographically.

Dental studies were reported by T. Kinersly et al. (2). The introduction gives a review of the application of lasers to biological systems as well as the principles of operation of laser systems. In this experiment a microscope and a system of lenses aligned on an optical bench were used to produce focused ruby laser beams.

The following oral specimens and dental materials were tested: hard tissues; powdered, sectioned and intact tooth enamel; calcified and decalcified dentine; cementum calculus and bone, carious and noncarious teeth that were naturally and artificially stained; soft tissues of the lip, tongue and oral mucosa in vivo and biopsy specimens of oral mucosa. Dental materials tested were amalgum, gold alloy, solder, matrix bands, orthodontic wire and acrylic plastic.

Chalky spots, craters or holes appeared in the impact areas of intact and powdered samples of dental tissues. Stains were easily removed from the surface of teeth. With impact spots of 20-25 μ holes 25 - 30 μ in diameter resulted in noncarious enamel and increased in diameter to 50 - 75 μ in carious areas.

An unfocused laser pulse of 0.5 joules to the lip produced no sensation of heat or pain. When such an area was stained, sensations of heat were reported. Herpes simplex of the lip was unaffected by a single exposure to the laser beam.

Dental materials were cratered. Attempts to weld metals were unsuccessful. The setting time of freshly mixed batches of pink acrylic were retarded.

The applications of laser beams to dentistry are discussed.

Extracted teeth and a molar tooth in vivo, were exposed to laser energy by L. Goldman et al (3). The energy density ranged from 4,000 - 13,000 joules/sq. cm. The teeth were sectioned and examined in normal light, polarized light and microradiographically.

Normal enamel showed pitting and charring in the impact area but microradiographic examination showed no changes in the subsurface enamel and dentine at energy densities of 13,400 joules/sq. cm. Enamel was penetrated 2.5 mm. with no changes seen in the enamel adjacent to the hole. At an energy density of 4,000 joules/sq. cm. a carious lesion was not deeply penetrated. Two impacts of 13,400 joules/sq. cm. to a superficial carious area produced deep penetration. Microradiographic and polarized light examination of sections indicated the carious area was not affected by the laser impact. Color increases absorption of laser energy by tooth structure.

Using fiber optics a molar was exposed to laser energy in vivo. The temperature rise as measured by thermistor placed along the side of the tooth was only 10°C and the patient reported no discomfort. On the bench this tooth recorded a temperature rise of 29°C from an exposure to laser beams of 4,000 joules/sq. cm./millisecond.

A microbeam probe capable of vaporizing biological materials is formed by focusing through a microscope. The sample is vaporized by the laser at 5,000°K. A crater about 50μ in diameter is produced and corresponds to a sample size of about 10^{-7}g.

The report by Rosan et al (4) discusses a spectral analysis of brain, pancreas, elastin, pearly calculus and hemorrhagic calculus. There was more iron found in the sample of hemorrhagic calculus compared to the pearly calculus.

A ruby laser was used by H. M. Goldman et al (5) to vaporize minute amounts of calcified tissues. The laser beam produces craters 50 μ in depth and 50 μ in diameter. Spectrographic analysis of supragingival and subgingival calculus and mandibular cortical bone is reported. These were analyzed for Mg. Al, Si, Ca, P, Fe, Cu and Zn. Themost noteworthy finding was the greater Ca content of supragingival calculus compared to subgingival calculus.

Carious teeth were exposed to the impact of a pulsed ruby laser with exit energy of 90 joules and focused energy densities of 9,000 joules/sq. cm., by L. Goldman et al (6) The teeth did not become hot on the outside even after repeated impacts. The effect on caries varied from the production of small 2mm. deep holes to the complete disappearance of the carious tissue. Areas of destruction were smaller but deeper with focused beams.

Laser beam energy of 5 - 20 joules and 2 ⇔ 5 joules was used in studies by Stern et al (7). Intact anamel showed a glass-like fusion with reduced birefringence under polarized light. Dentin was exposed to comparable energies which caused charring due to its higher organic content. The reflecting property of enamel causes considerable reflection of the laser beam. Restoration of silver and gold absorbed more of the laser beam than did adjacent enamel resulting in destruction of the restoration. Efforts to fuse

powdered substances to enamel with laser beams were handicapped by differentials in energy dissipation of the structures and by the impact power of the beam.

Studies on rat tissues were carried out by Taylor et al (8). The introduction reviews briefly studies of the interaction of laser radiation with biological tissues. With the interest in the application of lasers as an effective microsurgical instrument, the teeth and oral mucosa of small animals were studied grossly and microscopically after exposure tovarying intensities of laser radiation.

The authors describe in some detail the ruby laser used in their experiments which emitted laser energy at a wavelength 6,943 Å units. Twelve Syrian hamsters were used, six receiving 35 joules laser radiation and six receiving 55 joules of laser radiation directed at the anterior teeth and tongue. The spot size was 0.5 mm. the pulse duration 3 milliseconds. Animals were sacrificed at three and seven days and the tissues stained with hematoxylin and eosin, mallory and periodic acid - Schiff stain. Jaws were decalcified, sectioned at 12 μ and stained with hematoxylin and eosin.

The gross changes in tooth surface were the same as described by other authors. They were more severe following laser irradiation at 55 joules than at 35 joules. The tongue, exposed to 35 joules initially showed a reddish spot. By 3 days a large ulcer appeared. By seven days healing became evident. The changes in the tongue exposed to 55 joules were similar but more severe.

Microscopic findings in the pulp at three days following exposure to 35 joules of radiation showed necrosis of the pulp at the site of impact and hemorrhagic necrosis some distance along the incisor pulp. The pulpal changes were more severe following 55 joule irradiation. By seven days, signs of healing were evident in the group irradiated at 35 joules but not in the group irradiated at 55 joules. The tongue showed no specific inflammation at three days in the 35 joule group. After seven days there was evidence of granulation tissue and epithelization. There was reduced odontoblastic activity in the molar pulp and gingival inflammation in the molar region some distance from the impact area. The interdental septum showed decreased osteoblastic activity.

The preliminary findings appear to indicate there is scattering of the beam to the adjacent tissues.

The literature reviewed on the interaction of laser radiation with oral hard and soft tissues is of a preliminary nature, dealing with gross findings of short term experiments. The microscopic findings presented are also preliminary in nature in those cases dealing with human extracted teeth. There have been several in vivo experiments dealing with oral mucous membranes and teeth. In these cases the experiments were premature and dangerous since the interaction of laser radiation with oral tissues is not well understood. The only precaution taken was protection of the eyes. Since the possible side effects from exposure to laser radiation are not known, human experimentation at this time seems ill advised.

Although some in vivo information is presented in the paper by Taylor et al, this could have benefited from associated in vitro studies. The findings from the Mallory connective tissue stain and the periodic

acid - Schiff stain for mucopolysaccharides are not presented. The entire question of protective shielding and controlled therapeutic dosage has not ;yet been investigated. There are obvious hazards from the material scattered in the plume, which may be embedded in or injurious to adjacent oral tissues. Production of a crater during irradiation may tend to scatter the plume radiation through a small angle, thus causing a high energy density of scattered radiation within a specific oral region. Scattering of radiation and particulate matter through the hard palate to other anatomical regions probably presents a minimal hazard at these energy and power levels. Scattering of particulate matter through the pharynx and bronchi and into the lungs is presently a problem in high speed restorative dentistry. Lung abscesses are possible. Laser radiation may consequently present a greater hazard where the scattered material (in the plume) has a finite velocity. At higher energy and power levels, these problems may be of greater significance.

It must be recognized that the long term effects of trauma to oral tissues are similar to those in other anatomical sites. Should healing not readily occur, continued irritation with resultant pathological consequences may arise.

References

1. Lobene, R. R. and Fine, S. Interaction of Laser Radiation with Oral Hard Tissues. Presented at the International Association For Dental Research, July, 1965.

2. Kinersly, T., Jarabad, J. P., Phatak, N. M., and DeMent, J., "Laser Effects on Tissue and Materials Related to Dentistry," J.A.D.A., 70:593 , March 1965.

3. Goldman, L., Gray, J. A., Goldman, J., Goldman, B., and Meyer, R., "Effect of Laser Beam Impacts on Teeth," J. A. D. A., 70:601, March, 1965.

4. Rosan, R. C., Healy, M. K., and McNary, J. F., Jr., "Spectroscopic Ultramicroanalysis with a Laser," Science, 142:236, Oct. 1963.

5. Goldman, H. M., Ruben, M. P., and Sherman, D., "The Application of Laser Spectroscopy for the Qualitative and Quantitative Analyses of the Inorganic Components of Calcified Tissues," Oral Surg, Oral Med. and Oral Path., 17:102, Jan. 1964.

6. Goldman, L., Hornby, P., Meyer, R., and Goldman, B., "Impact of the Laser on Dental Caries," Nature, 203:417, July 1964.

7. Stern, R. H., and Sognnaes, R. F., "Laser Beam Effect on Hard Tissue," J. Dent. Res., 43:873, Sept-Oct, 1964 (Abstract).

8. Taylor, R., Shklar, G., and Roeber, F., "The Effect of Laser Radiation on Teeth, Dental Pulp and Oral Mucosa of Experimental Animals," Oral Surg., Oral Med. and Oral Path., 19:786, June 1965.

Entomology

In studies by Wilde (1), a non-Q-switched ruby laser (pulse duration 2 milliseconds, output energy 0.57 joules) and a Q-switched ruby laser (pulse duration 10^{-7} sec. energy 0.06 $\pm$0.04 joules) was used. The variability in pulse duration was obviously high. The impact areas were 1.5 millimeter; irradiation distances, 38 millimeters to 30 centimeters. Larvae of trogoderma versicolor species of dermestid beetles were irradiated. Three were radiated behind the head region and died within 3 to 48 hours. Irradiation of the dorsal and ventral abdominal areas resulted in loss of negative phototaxis, cessation of feeding and death of all larvae within 18 days. When directed at the posterior dorsal surface no loss of coordination, mobility, or negative phototaxis occurred but it did affect the epidermal layer as shown by succeeding molts. Epidermal layer damage was transmitted through 3 molts in 4 of the treated larvae. Pupae or adults did not exhibit this type of injury. Cockroaches were irradiated in various regions. Life duration was reduced. The author concludes irradiation may be considered for insect control, particularly where monolayering of food deposits occur.

The approach considered is expensive compared to possible chemical approaches, or the use of incoherent radiation. No immediate, obvious value in pest control is evident, unless sterility can be easily produced in some insects. The technique of release of insects made sterile by other types of radiation among the normal insects has been attempted as a means of insect control. Although there is no justifi-

cation for the use of pulsed ruby laser radiation, the advent of gas lasers operating at shorter wavelengths may deserve investigation in this respect.

Other studies by Witt, Reed, and Tittel on the behavior of irradiated spiders, particularly with respect to web construction are discussed in "The Laser Microbeam" section of this report. In the report by Goldman et al, "Pathology of the Effect of the Laser Beam on the Skin," one black widow spider, two cockroaches and three caterpillars were irradiated with a pulsed ruby laser at an (undefined) energy level. Hematoxylin and eosin stained sections of the caterpillar showed sharply demarcated areas.

References

1. Wilde, W.H.A., "Laser Effects on Two Insects, "The Canadian Entomologist, 97:88, 1965.

2. Witt, P.N., Reed, C.F., and Tittel, F.K., "Laser Lesions and Spider Web Construction," Nature 201 (4915):150, 1964.

3. Goldman, L., et al, Nature 197 (4870):912, Mar. 1963.

Mechanisms for Laser Interaction with Biological Systems

Elucidation of the interaction of laser radiation with biological systems is a complex problem depending on many physical and biological parameters. Knowledge is required of the characteristics of the laser radiation itself (wavelength, power, energy, energy density, polarization, and some aspects of coherency), the physical characteristics of the biological system (absorption coefficients, scattering coefficients, thermal diffusivity, specific heat etc.), biological characteristics and reactions of the system (metabolism, age, pathological characteristics, anatomical structure, biochemical structure, etc.) and the possible variation of both physical and biological characteristics during laser irradiation (bleaching, coagulation, volatilization, physical rupture, alteration of function, etc.). The mechanisms by which electromagnetic laser energy is transformed to thermal energy, mechanical energy, or secondary radiant energy (wavelength alterations, scattering) require at least partial knowledge of physical and biological characteristics listed above. In this section, a review will be presented of the studies carried out to characterize and elucidate mechanisms of laser energy transformation in biological systems. It must be noted that while the transformation to thermal energy is most commonly observed and is a prerequisite for some secondary effects, it is not the only transformation that occurs nor is it always of primary importance as to the resulting biological effects on a short or long term basis.

A. Modes of Thermal Energy Conversion

1. Theoretical Models.

Burkhalter (1) has studied the interaction of laser radiation with various physical materials. He has given attention to heating during and following laser interaction. Models which are intended to correspond to the absorption of focused and unfocused Q-switched pulses are presented. Solutions to the heat flow equation (eqn 1),

$$\frac{\partial T}{\partial t} = D\nabla^2 T \qquad \text{(eqn. 1)}$$

where

T = temperature increase above ambient

t = time elapsed since the instantaneous laser pulse

D = thermal diffusivity of the material (this constant essentially determines the rate of heat conduction).

are obtained with the following assumptions:

1. Laser energy is delivered in an instantaneous pulse.
2. For the focused case, energy is absorbed by the medium only at the focal point.
3. For the unfocused case, energy is absorbed only at the surface of the material.
4. The thermal constants of the material do not change during or following the laser pulse.

Relatively simple solutions to the heat flow equation can be obtained under these assumptions. These solutions are given by Burkhalter as:

$$T(r,t) = \frac{Q}{\rho s (4\pi Dt)^{3/2}} e^{-\frac{r^2}{4Dt}} \quad \text{(focused case)}$$

$$T(z,t) = \frac{2Q}{A\rho s (4\pi Dt)^{1/2}} e^{-\frac{z^2}{4Dt}} \quad \text{(unfocused case)}$$

where

r = radial distance from laser focal point

z = depth below absorbing surface

A = cross sectional area of beam at absorbing surface (note Burkhalter does not discuss the fact that equation 3 holds only when,

$$\sqrt{\frac{A}{\pi}} \gg z$$

P = mass density of material

s = specific heat of material

Q = number of calories absorbed at surface or at focal point.

Burkhalter states that these assumptions correspond to a rough model for absorption of focused and unfocused Q-switched laser pulses. Burkhalter does not present data justifying the applicability of this model to biological systems. One can, however, show from simple considerations that it is improbable that pigmentation in biological tissue occurs in layers so thin and opaque as to warrant the assumption that extremely short, (nanosecond range) laser pulses are required to avoid heat conduction during the laser pulse.

Ham has noted that the darkly pigmented human pigment epithelium absorbs approximately 40% of incident ruby laser radiation in a layer

10 microns thick. If one assumes such tissue to be composed of densely packed, non-scattering melanin granules, then the absorption of light in pigment epithelium should obey the following relation

$$\frac{E \text{ absorbed}}{E \text{ incident}} = 1 - e^{-\alpha x}$$

where

x = thickness of pigment epithelium (cm)

α = absorption coefficient (cm^{-1}).

If human pigment epithelium is assumed to be 10 microns thick then

$$0.40 = 1.00 \ (1-e^{-\alpha 10^{-3}})$$

This relation yields an approximate absorption coefficient for melanin of $\alpha = 500\ cm^{-1}$. This result indicates that a layer of melanin 20 microns thick is required to absorb a large fraction ($\approx 70\%$) of collimated incident laser radiation. Assuming that such a layer exists in biological material and that all incident laser radiation is absorbed in this layer, it becomes a simple matter to calculate approximately how short a laser pulse must be for negligible heat conduction to occur during the pulse. The laser pulse duration, τ, must be less than $\frac{x^2}{4D}$ for negligible heat conductions to occur during the laser pulse. If $x = \frac{20\mu}{2}$ and D is approximately the same as the diffusivity of water ($1.4 \times 10^{-3}\ cm^2$ per second) then τ must be less than 0.2 msec. Therefore, in most biological tissue layers, absorption coefficients are not high enough to warrant the assumption that Q-switched (nanosecond range) laser pulses are necessary for negligible heat conduction to occur during the pulse. However, Q-switched (nanosec) laser pulses may be necessary for negligible heat conduction to occur within the pigment granule during the pulse. This

may be significant in elucidating the difference between threshold values for Q-switched and non-Q-switched irradiation.

For focused laser radiation, spot diameters of one micron are achievable. However, insufficient light abosrption (≈ 5%) will occur in a one micron diameter, spherical volume to warrant use of the Burkhalter model. Heat flow will not take place in these focal diameters for laser pulse durations of less than 0.4 μsec.

In summary, experimental evidence shows that absorption in biological materials is normally too low to require the use of a Q-switched model for laser radiation absorption. However, as Burkhalter points out (1) at high peak power densities, absorption may become non-linear and increase. It may be significant in pigment granule absorption.

Studies by Yura (2) on laser interaction with metals, particularly the transition metals, have given evidence for long electron recombination lifetimes as discussed by Burkhalter. In these studies, it is suggested that excited electrons are trapped in metastable quantum states and subsequently recombine emitting light and/or imparting vibrational energy (phonon production) to the metal lattice. Phonon production is the mechanism by which heat is generated within the metal. Electron recombination lifetimes from such metastable states are predicted to be of the order of one nanosecond, which is comparable to the pulse duration of a Q-switched laser. Burkhalter states "in this manner, a material may absorb much more energy per unit volume from short pulses such as Q-switched pulses than from long ones." One can see, however, that lower electron energy states may be depopulated by a Q-switched pulse, and relatively few electrons will decay back to these states during the pulse.

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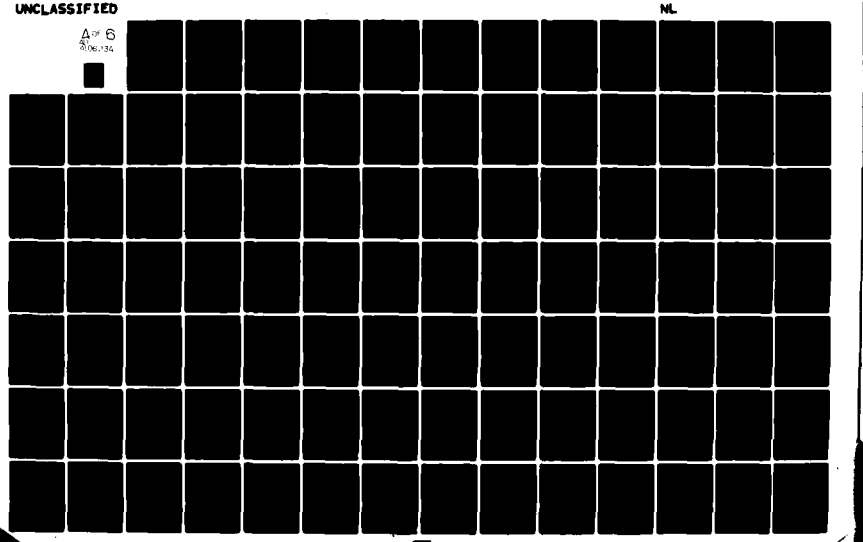

In this case, radiation absorption may actually decrease rather than increase due to the depopulation of lower electron energy states. Other quantum state configurations exist where increased absorption may occur for high photon densities and/or short laser pulse durations. It is possible, however, that Q-switching may result in a non-linearly decreasing absorption coefficient rather than a non-linearly increasing absorption coefficient. The electron trapping mechanism discussed by the author is an energy storage process. A comparison between this type of energy storage and classical thermal energy storage is developed in the following paragraphs for biological systems.

Consider a highly pigmented tissue layer (eg. the retinal pigmented epithelium) which is heated by a laser pulse with uniform energy density across a collimated beam. Assume that one wishes to raise the temperature of the adjacent retinal tissue (photoreceptors) to some critical temperature, T_c, Without considering quantum processes, it is apparent that laser pulses of duration shorter than the time required for heat to conduct across a single layer of melanin granules in the pigment epithelium (assume melanin granules 0.3μ in diameter) will serve only to store thermal energy in the melanin. Therefore, to raise the temperature of the adjacent retinal tissue to T_c will require that more radiant energy be supplied as the laser pulse duration is shortened. Conversely, if the laser pulse is too long, heat will conduct away from the pigment epithelium too rapidly to raise the temperature of the adjacent retinal tissue to T_c. If thermal energy is stored in a single layer of melanin for a time approximately equal to the laser pulse duration then the laser energy that must be

supplied to result in steam production is a minimum compared to longer or shorter pulses (4). The time for heat to conduct across a single melanin granule may be of the order of nanoseconds, based on the thermal diffusivity of water. Hence, thermal energy can be stored in a melanin granule, with relatively little loss, during a Q-switched laser pulse based only upon classical heat flow considerations. If the time required for phonon production in biological material, such as melanin is also of the order of nanoseconds, then it will become difficult to separate classical heat conduction effects from the quantum effects (electron trapping) that Burkhalter has cited. Q-switched pulses to the retinal pigment epithelium should also be the most efficient pulses for producing high temperatures in the retina from the standpoint of heat conduction alone. Hence, some of the drastic effects noted following Q-switched laser irradiation of biological systems may be explained in terms of classical thermal conduction. Further experimental and theoretical work is required before the importance of quantum mechanical interactions per se during Q-switched laser impact is known.

Fine et al (5) have included the effects of interfaces on laser radiation absorption in biological systems.

> "Biological systems may be considered to consist of strata of differing composition and properties, separated by interfaces. For example, in the abdominal region, the strata can be grossly considered as skin and discontinuous subcutaneous tissue, muscle, peritoneal layers, and viscera. Interaction with this multilayered system will differ from interaction with a homogeneous system. Multiple reflections can occur from the various interfaces, on both a gross and microscopic level, resulting in trapping of radiation energy within a specific gross or microscopic region, particularly if a change in wavelength occurs. The energy density within this region can consequently be greater than if the region were not part of a multi-layered system and if reflections, particularly from the interface deep to the layer, did not occur."

Calculations are performed on infinite, three-layer material (App. 1)(5) with partially reflecting interfaces and a given percent absorption in the central layer. It is shown that the radiant energy absorbed in the central layer is increased in the presence of partially reflecting interfaces although the energy absorbed in the central layer cannot be greater than twice the energy absorbed without boundaries. Greater absorption can occur in the central layer if there is an alteration in wavelength with an associated alteration of absorption coefficient. If there is an alteration of wavelength because of interaction at the interfaces, then considerable trapping of energy within the central region could occur (the greenhouse effect).

Fine et al suggest that increased absorption at abdominal interfaces may result in steam production and possibly cause the skin distention that has been observed in mice (5). An extension of the interface model should include the effect of tissue curvature during the distention. Reflective focusing of trapped radiation will increase the energy density of the radiation in zone 2. Since the curvature of the interfaces changes during laser impact, the problem of reflective focusing in skin distention is both non-linear (due to non-uniform steam production) and time varying. Further modification of the simple model presented by Fine et al should include the effects of refractive focusing of incident laser light by both external lens systems and by the curvature and refractive indices of the various tissue layers. Models are in preparation, by the authors of this review, which include the effects of refractive and reflective focusing of laser radiation in biological tissue (6).

Variations in refractive index on a cellular scale introduces radiation scatter. More refined models of laser radiation absorption should include this effect.

In the excellent gross and histological studies by Helwig et al (7) the epidermal temperature elevations for various beam diameters and incident energies delivered by a non-Q-switched ruby laser have been calculated. Pertinent parameters for skin are taken from studies done at low power levels such as that of a spectrophotometer or carbon arc and on various animals and in vitro human systems by Hardy et al (8), Davis (9), Henriques and Moritz (10), and Kupenheim et al (11). For beam diameters of the order of 1 cm and an epidermal thickness of about 100μ, heat conduction can be ignored during a laser heating pulse of 1.6 msec duration in order to obtain a rough estimate of the temperature elevation of the epidermis. The equation used by Helwig et al is:

$$T = \frac{E \alpha t \ (1 - r)}{\frac{\pi d^2}{4} \alpha t \ \rho C}$$

where E is energy of the incident laser beam in calories; α, the absorption coefficient at 7,000 Å in cm^{-1}; t, the epidermal thickness; (1-r) the fraction of the beam not reflected; $\frac{\pi d^2}{4}$, the exposure area or image size; ρ, the epidermal density in g/cm^3; and C, the epidermal specific heat in cal/g per °C. The factor αt in the denominator is probably an approximation to the expression $(1 - e^{-\alpha t})$ for αt small. For $\alpha \doteq 20\ cm^{-1}$ and $t \doteq 100\mu$, the error in using this approximation is about 10%. Helwig's calculations indicate that the heat content per gram ($\approx 5 \times 10^{-2}$ cal/gm) necessary for protein denaturation is easily

achieved in the epidermis by laser irradiation in the ten joule range for beam diameters of about 1 cm (Fig. 23 of (7)). The authors conclude that thermal processes, such as protein denaturation, are sufficient to explain laser induced lesions. Furthermore, they feel that the magnitude of the predicted thermal effects per se would tend to limit the detectability of other physical or biologic effects. Further attention should perhaps be directed to the photographs shown by Helwig et al of histological slides taken of porcine skin following laser irradiation in the 12 joule range. These photographs show possible marked mechanical rupture (perhaps due to steam) of the epidermal layers which may be different from thermal effects per se. However, other possible explanations such as formation of an exudate are possible. Whether one should include pressure transients cratering, change of phase, and ablation as "thermal effects" requires further consideration.

In the course of their comprehensive well documented irradiation studies on the eye, Ham et al (12) have developed a model for laser radiation absorption and heat conduction in the human ocular fundus. According to this model, significant absorption begins at the pigment epithelium where approximately 40% of the laser radiation is absorbed exponentially in passing through 10 microns of tissue, and approximately the remaining 60% is absorbed exponentially in passing through 100-150 microns of choroid.

In Ham's model, absorption in the retina and anterior ocular media is considered as negligible. This is a reasonable assumption since absorption and/or scattering in these regions is only about 10% at 6943 Å. Other

highly absorbing tissues, such as the iris, are not specifically included. It is also reasonably assumed, only for the purposes of this model, that pigmentation is uniform in the pigment epithelium and choroid. Scattering is mentioned as a possible attenuation mechanism in the fundus, but is not considered in the model, because of its complexity. In the lightly pigmented choroid, some thought can be given to back-scattered radiation from the sclera which may yield higher radiation energy densities at the pigment epithelium.

Ham et al note that the highest temperature gradients will occur between the pigment epithelium and retina, due to the relative transparency of the retina. Although the retina is transparent to visible wavelengths, it may be relatively opaque to those associated with heat. This high temperature gradient will cause heat to be conducted preferentially to the retina rather than to the choroid. A more complex model than that presented may include the effects of choroidal blood supply on radiation absorption and heat dissipation. Although the velocity of flow in the choriocapillaris is low, the blood volume may be high, and merit consideration. The highest temperature will, as indicated in the model, occur initially in the pigment epithelium and spread via heat conduction to the photoreceptors in the retina.

A reasonable model is assumed by Ham et al in which the maximum temperature of the irradiated portion of the pigmented epithelium is estimated by neglecting thermal conduction and considering a "pill box" ten microns thick and having a diameter the same as the laser beam incident at the pigment epithelium, If 40% of the radiant energy incident on the

surface of the pill box is absorbed, then the authors conclude that a radiation dose of 0.85 J/cm^2 (delivered in 200 μsec) yields a threshold lesion in the rabbit retina and will produce a temperature rise of approximately 81°Centigrade in the pigment epithelium (12).

Examination of this calculation shows that Ham has assumed a relation for temperature increase of the form:

$$T = \frac{H}{t}$$

where H is the number of calories absorbed in the pill box per unit cross-sectional area of the incident laser beam (at the pigment epithelium), and t is the thickness of the pill box. The specific heat and density of the pigment epithelium are assumed to be unity (the same as that of water).

Ham et al have considered that radiation absorption in the pigment epithelium and choroid would probably occur exponentially. Another possibility, consequently, is to assume exponential absorption (diathermancy of the tissue). This would then yield a somewhat higher expected maximum temperature in the pill box.

The following relation assumes adiabatic walls (no heat conduction out of the pill box), zero reflectivity at the pigment epithelium, no scattering and zero conductivity within the pill box based on the model by Ham et al. If the diathermancy of the tissue is included via the absorption coefficient, α ,

$$T(x)_{max} = \frac{E_o \alpha e^{-\alpha x}}{4.18\, DC_p\, A}$$

$T(x)_{max}$ is the maximum temperature rise at a depth, x, within the pill box; E_o is the incident energy from a collimated beam at the retina; α is the absorption coefficient for the pigment epithelium (approximately 500 cm^{-1}); A is the cross-sectional area of the beam; D and C_p are the density and specific heat of the pigment epithelium and are assumed to be approximately unity. For E/A = 0.85 joules/cm^2, T (0), the maximum surface temperature rise of the pigment epithelium nearest the retina is 102° C, of the same order of magnitude as the 81° C calculated by Ham. For the Q-switched case of 0.07 joules/cm^2 incident on the retina T(0) is 8.4° C, and varies little from the 6.7° C as Ham has calculated, if the assumption is made that radiation absorption in the pigment epithelium and choroid will probably occur exponentially, as discussed by Ham et al. The maximum temperature of the pigment epithelium under adiabatic conditions (no heat flow) will therefore be somewhat higher than that obtained for the model where uniform absorption occurs. The assumption of exponential decay leads to temperatures above the steam point for water when ambient temperature (approx. 37° C) is added to the 102° C temperature rise for the case of 0.85 j/cm^2 incident at the retina. In the extensive investigations by Ham et al, this energy density, when delivered in a 200 μsec ruby laser pulse, resulted in an ophthalmoscopically visible lesion in the fundi of pigmented rabbits five minutes after exposure. This type of lesion was termed a "threshold lesion" by Ham. Ham points out that such a lesion may, however, not represent minimum irreversible damage to the rabbit retina. Further study is required before the importance of steam production in retinal damage can be ascertained.

Additional data is presented byHam et al., based on extensive studies for exposure times longer than 200 μsec (using Xenon or carbon arc light sources) which tabulates the energy density required for a threshold lesion and the total temperature rise of the pigment epithelium at the end of the pulse. Temperature calculations are presented for cases where first, heat conduction is neglected and second, where heat conduction from the faces and sides of the "pill box" absorber in the pigment epithelium is taken into account. Calculations were carried out by Ham et al on the model and are presented in Table I.

TABLE I

Maximum temperatures in the PE for different exposure times as estimated on the basis of the pill box model. An electrical analog has been used to take account of heat conduction in the model.

Exposure time in μsec	Radiation dose in j/cm^2	Max. temp. rise in PE neglecting conduction	Max. temp rise in PE correcting for conduction
0.030	.07	6.7	6.7
200.0	.85	81.0	52.8
500.0	1.10	105.0	50.5
1,000.0	1.38	132.0	55.0
2,000.0	1.72	165.0	53.5
5,000.0	2.41	230.0	49.3
10,000.0	3.07	294.0	47.3
20,000.0	3.90	373.0	46.8

In the Q-switched case, temperatures of approximately 45°C can be expected (assuming exponential or uniform absorption). The relatively low temperatures produced by Q-switched laser irradiation and the appearance of choroidal hemorrhage extending into the subretinal spaces when only relatively mild retinal damage is seen lends credence to the hypothesis that other than thermal processes per se may account for Q-switched laser damage to the rabbit eye and other biological tissue (12,5).

In development of the model, uniform absorption in the subretinal pigmented tissues was assumed by both Ham (12) and Vos (13). They have not included the problems of intra-layer and interlayer inhomogeneities of pigmentation, possible laser emission inhomogenieties across the end surface of the rod, and the effects of radiation scatter. It should be noted that these inhomogeneities have been considered by Ham et al in their comprehensive studies. Attention to the problems of interlayer variations in radiation absorption by biological tissue has been given by T.P. Davis (9) and J.M. Davies (15) in investigations of *in vivo* burn production in porcine skin from broad-band radiation. Davies (15) has given some consideration to the problems of scatter in tissue. Felstead and Cobbold (16) have developed an electrical analog for thermal conduction in the human eye. They assume rotational symmetry of the eye and coagulating beam and neglect effects due to vaporization and pressure. Some consideration of the relative absorption of light in the various tissue layers is given, but, diathermancy is neglected insofar as these layers are assumed to absorb uniformly as a function of depth. Radial variations in beam intensity can be included in the Felstead-Cobbold analog if these variations are invariant under rotations about the optical axis of the incident beam. Such variations are not considered by Felstead and Cobbold in using the analog.

A number of attempts have been made to measure temperatures at various sites in rabbit eyes. These studies, in some cases, have been carried out in an attempt to verify various theoretical models for thermal alterations in the rabbit fundus and to elucidate mechanisms of laser eye damage and clinical photocoagulation of the *in vivo* human eye.

Since direct temperature measurements are extremely difficult to perform during and following laser impact, and are probably not reliable, expected temperature elevations in the ocular fundus must be calculated, (as they have been done by Ham et al) from measurements of laser energy, laser pulse duration, beam diameter at the pigment epithelium, absorption of various ocular tissues, and the physical dimensions of the tissues involved. Beam splitter techniques for measuring laser output energy and pulse duration must be carefully designed to avoid the problems of inconsistent beam polarization. The measurement of laser beam diameter within the eye required accurate knowledge of the optical properties of the particular eye under investigation. For example, considerable differences may be present between the human and rabbit eye. The absorption properties of various ocular tissues have been considered by Geeraets et al. The figures of 40% and 60% attenuation at 6943Å in the pigment epithelium (PE) and choriod, respectively, must take into account the fraction of the light absorbed and the fraction scattered. Variations in pigment epithelium and choroidal thickness and density of pigmentation are important, but difficult to include in a model. All of these factors make it very difficult to predict an accurate temperature distribution within the eye following laser irradiation. An error analysis, accompanying the calculations which are intended to predict these temperature distributions would be of interest. Further correlative efforts between more accurate temperature measurement and theoretical prediction are necessary before a model for thermal interaction in the eye can be developed.

Vos has performed a theoretical analysis of experimental data obtained by Ham et al (14) in studies of retinal flash injuries produced by white light in rabbits (13). In the Vos analysis, a model for steam production in the pigment epithelium is developed and compared to a model where temperature elevation per se is considered as the mechanism for irreversible

threshold lesion production in the rabbit pigment epithelium. Temperatures well above the steam point for water, are calculated for the energy densities and irradiance pulse durations used in the studies by Ham. Based on these results, curves of constant steam production (by weight) are drawn and found to coincide reasonable well with Ham's experimental results for exposure times between 20 msec and 250 msec. Vos interprets these results in the hypothesis:

> "A threshold lesion as defined by Ham is produced when a steam explosion takes place which is sufficient to give a marked disruption of the retinal tissue. In very small images, this may be expected to happen when steam production reaches an absolute minimum. In large images, the production of steam per unit of area should be the determining factor." (13)

This hypothesis should be subject to review, based on the excellent experimental results of Ham et al. using relatively monochromatic ruby laser radiation in the one millisecond and 30 nanosecond range.

Vos' calculations of temperature elevations are indirect and rely on experimental data and associated variation similar to those that were pointed out in conjunction with the Ham model of the rabbit fundus. Vos also neglects the diathermancy of the pigmented tissues posterior to the non-pigmented retinal layers. It is assumed that all of the radiation incident at the retina is uniformly absorbed in a volume of pigmented tissue termed "the thermal image" by Vos. The depth of the thermal image is neglected for exposure times longer than 300 μsec whereas the cross-sectional area is assumed to be that of the incident beam at the retina. However, for exposure times greater than 1 μsec and less than 300 μsec, the depth of the thermal image must be included. Therefore, if the Vos model is to be used to predict the temperature distribution within the "thermal image", all dimensions of the thermal image should be considered. Should this task be pursued, a model for the ocular fundus including radiation scattering and tissue diathermancy is desirable.

In development of the model, uniform absorption in the subretinal pigmented tissues was assumed by both Ham (12) and Vos (13). Not considered here are the problems of intra-layer and interlayer inhomogeneities of pigmentation, possible laser emission inhomogenieties across the end surface of the rod, and the effects of radiation scatter. It should be noted that these inhomogeneities have been considered by Ham et al in their experimental studies. Attention to the problems of interlayer variations in radiation absorption by biological tissue has been given by T.P. Davis (9) and J.M. Davies (15) in investigations of in vivo burn production in porcine skin from broad-band radiation. Davies (15) has given some consideration to the problems of scatter in tissue. Felstead and Cobbold (16) have developed an electrical analog for thermal conduction in the human eye. They assume rotational symmetry of the eye and coagulating beam and neglect effects due to vaporization and pressure. Some consideration of the relative absorption of light in the various tissue layers is given, but, diathermancy is neglected insofar as these layers are assumed to absorb uniformly as a function of depth. Radial variations in beam intensity can be included in the Felstead-Cobbold analog if these variations are invariant under rotations about the optical axis of the incident beam. Such variations are not considered by Felstead and Cobbold in using the analog.

A number of attempts have been made to measure temperatures at various sites in rabbit eyes. These studies, in some cases, have been carried out in an attempt to verify various theoretical models for thermal alterations in the rabbit fundus and to elucidate mechanisms of laser eye damage and clinical photocoagulation of the in vivo human eye.

Campbell, Noyori, and Rittler have studied the temperature elevation at the interior surface of the retinae of <u>in vivo</u> grey chinchilla rabbits during and following laser retinal coagulation. The laser photocoagulator (17) utilized a ruby crystal and suitable viewing system for the observation of coagulations and thermocouple positioning. Laser output was maintained at 0.065 joules for pulse durations of one millisecond or less. Baldwin-Lima-Hamilton, micro-miniature, exposed junction, chromel-alumel thermocouples were inserted through small scleral incisions and positioned with the active junction assumed to be in thermal contact with the interior surface of the retina. For temperature measurements at the site of the coagulation a curved thermocouple was inserted through the sclera behind the area to be irradiated thus eliminating any shadowing of the retina by the thermocouple during irradiation. Temperature measurements outside the coagulated area were made by inserting the thermocouple in a position tangential to the surface of the retina. Thermocouple output was displayed on an oscilloscope.

Results indicated that temperature changes of less than 0.5 degrees centigrade occurred in the plane of the retina at distances greater than 1 millimeter from the coagulation site. At the site of the coagulation itself temperature elevations of 30 degrees centigrade were recorded. Temperature changes of about 0.5 degrees centigrade were obtained with the thermocouple tip positioned in the middle of the vitreous and centered in the laser beam.

There are errors associated with thermocouple measurements that are made when the temperature sensing junction is exposed to direct or scattered laser radiation (20,21). The oscilloscope traces that are illustrated in the report by Campbell et al were recorded on a very slow time scale. Thermocouples of this type have been shown to respond in approximately one millisecond when exposed to direct or scattered laser radiation (20). Therefore, much important fine structure such as thermocouple irradiation is lost in oscilloscope traces where the time scale is of the order of seconds per centimeter. It is doubtful that Campbell et al could observe the effects of direct thermocouple irradiation from direct or scattered light in their oscilloscope traces. It should be noted that an irradiated thermocouple junction (and insulating sheath) can heat surrounding tissue. The temperature of the heated tissue is relatively persistent, having a thermal time constant of several seconds. The thermocouple outputs observed on slow oscilloscope traces by Campbell et al are probably a result of both direct laser heating and thermocouple heating of retinal tissue adjacent to the thermocouple junction. These two mechanisms of tissue heating require further consideration.

Measurements of the relative transmission characteristics of the in vitro human fundus have indicated that little energy is absorbed or scattered by the non-pigmented retina itself (22). Most of the absorption or scattering of ruby laser radiation takes place in the pigment epithelium and choroid posterior to the retina. Ham (12) assumes that the highest temperature elevations would occur when the retinal pigment epithelium absorbs and does not scatter incident radiation. Ham has also suggested that, from a practical standpoint, a temperature of close to or perhaps

at 100°Cin the pigment epithelium is required to coagulate retinal tissue to the point where the relative reflectance of light from the pigment epithelium through the normal retina and the retina with lesions cannbe distinguished by ophthalmoscopic observation. Vos (13) has suggested that temperatures achieved in the pigment epithelium reach the steam point and steam production accounts for lesions observed in the rabbit retina by Ham and co-workers. In either the Ham or Vos model, it is not necessary that temperature at the inner surface (anterior surface) of the retina will probably produce ophthalmoscopically visible retinal lesions. Further experimental study is required before the "temperatures" measured by Campbell et al. at the anterior retinal surface can be correlated to temperatures in the pigment epithelium. Measurements of this latter type should provide insight toward elucidating the mechanisms involved in clinical retinal coagulation and retinal damage from laser radiation.

Small temperature changes were noted by Campbell et al. outside the coagulated area. The mechanisms for these changes require further consideration. Some of the mechanisms may include scattering of light or thermal conduction. The thermocouple responses shown for points 1 mm outside the coagulated retinal site show rise times of much less than one second and decays of the order of a half second. If the retina is assumed to have the thermal diffusivity of water (1.4×10^{-3} cm^2/sec), then the time for conduction from the coagulated area to a point 1 mm from the edge may be longer than the time delay observed. However, analysis is difficult since the relationship between temperature distribution and coagulation of tissue is not well understood.

If thermal conduction is to account for temperature elevation at these sites, the thermocouple trace should show rather slow rise times (of the order of seconds) and a relatively persistent d.c. thermocouple voltage output at subsequent times. The very fast, transient thermocouple output noted by Campbell et al. could be due to scattered laser light reaching the thermocouple or to specific monitoring circuitry whereby the thermocouple output was differentiated.

The author concludes that thermal summation does not result with repeated coagulation, derived from his experimental studies of rapid temperature decay. Further temperature measurements are necessary in the pigment epithelium. Temperature measurements may be perturbed by direct or scattered laser radiation to the thermocouple. Further investigation where thermocouples are not exposed to direct or scattered laser radiation is necessary.

Najac et al (23) have carried out extensive thermocouple measurements in the moderately pigmented retina of gray chinchilla rabbit eyes (in vitro) during irradiation by a modified Meyer-Schwickerath light coagulator in the spectral ranges 380-750μ and 380-1,350μ. This work was a light coagulation study. The sensing junction was placed anterior to the pigment epithelium. Information as to the exact location of the thermocouple would be desirable, but, of course, is limited by biological factors. The temperature elevations measured for a threshold lesion (small amount of coagulation in the center of the lesion) ranged from 12.5° to 20.6° centigrade. These temperatures are cited as being below the steam point but high enough to produce albumin coagulation. This conclusion

may be correct at the point of temperature measurement but does not exclude steam production within the pigment epithelium itself.

The time delay between thermocouples placed at various locations within the retina were correlated with times for thermal conduction. For identical thermocouples, this technique would yield valid results. Data as to the response characteristics of the individual thermocouples and circuitry would have been of interest. Interchanging thermocouple positions between experimental runs and comparing the results might assist in decreasing effects due to dissimilarities between thermocouples. The usual problems of effects of direct and scattered light on thermocouple functions must be considered.

The problems of direct thermocouple irradiation have been discussed by Geeraets and Ridgeway (21), Nowak et al (20), Davis (28) and Crowder (24). Geeraets and Ridgeway have noted that the insertion of a thermocouple into biological tissues gives rise to artifacts of reflectance, absorption, and conduction, at the site in the tissue where measurements are being carried out. The relative size of the thermocouples with respect to the image size of the incident light beam on the retina and with regard to the dimensions of the biological structures is of great importance (21).

Crowder (24) has noted that a large artifact was introduced into measurements of the temperature of the vitreous at various points along the path of the beam during photocoagulation if a thermistor probe was placed directly in the photocoagulator beam. The detector absorbed a

significant amount of the light energy even when a fine mirror surface was used. To overcome this difficulty, Crowder has introduced a device whereby the thermistor probe is mounted at the tip of a fine needle and injected into the vitreous at various times following photocoagulation. While no calculations were shown, Crowder feels that the temperature of the vitreous during photocoagulation could be calculated from the decay curve of the thermistor. The photocoagulations used by Crowder were either one or five seconds in duration. The thermistor probe had a rise time of 0.085 msec and was injected in less than 10 milliseconds following the end of the photocoagulator pulse. When the coagulating beam diameter is less than one millimeter then appreciable heat flow can occur during the irradiance pulse. Unless the temperature at the point of measurement varies monotonically in time during the irradiance pulse and during the time before the thermocouple is injected, it is not possible to reconstruct a time temperature history from the decay curve of the thermocouple.

Attempts to reconstruct time-temperature histories from thermocouple decay curves have also been made by McCartney and Hall (25). Electronic, time-delay circuitry was thought sufficient to allow thermocouples implanted in adenocarcinoma tumors (in black mice) to come to thermal equilibrium and allow extrapolation of the thermocouples responses to yield temperatures during laser impact (25). Effects of direct thermocouple irradiation require evaluation. The results of extrapolations to zero time require further study.

A theory for reconstructing initial temperature distributions from data obtained at later times and at various positions has been presented by Masket (26) using a modified Green's function approach to the solution of the heat flow equation with time reversal. One must also consider the fact that if thermal injury or change occurs within a biological system during radiant insult, the chances are in favor of the system becoming non-linear as biological change proceeds. This further complicates the problem of reconstructing the time-temperature history of a biological system from data taken at times following thermal change.

Nowak et al (20) have shown, using the energy-specific heat balance equation, that a chromel-alumel thermocouple positioned in air and in the path of a 12J, 1.0 cm diameter, 1 msec laser pulse will indicate temperature rise of approximately 800°C. The thermocouple bead is assumed to have an effective thickness of 34×10^{-4} cm (4/3 the radius of the thermocouple bead) and a spectral absorptivity of 0.75.

To test these calculations, experiments were carried out whereby chromel-alumel thermocouples (30) with junction-tip diameters of approximately 0.002 inch and sheath diameters of 0.014 inch were exposed to unfocused radiation from a non-Q-switched ruby laser. The thermocouple was connected directly to the d.c. input of a Fairchild 704 oscilloscope. The response of the unshielded thermocouple is shown (20) with tip exposed in air perpendicular to a 12J beam. Also shown is the output of the thermocouple with exposed tip, but shielded sheath. A peak pemperature of 790°C was obtained in a good agreement with the previous calculation. Shielding the tip, or equivalently, shadowing the tip by the thermocouple sheath from the incident radiation, resulted in a modified response.

To evaluate further the response of the thermocouple, experiments were carried out at 18J in air, and at 12J in a 1% saline solution and in tap water. The solutions were contained in a plexiglass box 5 inches X 2 inches. The thermocouple was centered within the box.

The ratio of the peak temperatures, 1260°C and 790°C, obtained for 18 and 12 joule exposed-tip irradiations in air is in reasonable agreement with the ratio of the energies and with the calculations. The temperature of the thermocouples (1260°C and 790°C) is obviously of a different order of magnitude than the actual air temperature in the laser beam without the thermocouples present.

Thermocouple temperatures were measured within the solutions irradiated at 12 joules. No differences in response were noted between water and saline solution. With either the whole thermocouple exposed, or tip alone exposed, the peak temperature rise recorded was approximately 500°C. The peak of shielded-tip response in solutions, was 90°C. This is apparently due to scattered radiation reaching the tip; the irregularity of this peak should be noted. The expected rapid quenching of the tip when in solution was observed, i.e. 2 msec compared with 16 msec to 1/e of peak. The delayed peak in the shielded-tip response is due to the time required for the heat absorbed by the sheath to diffuse to the tip. This diffusion probably takes place along the thermocouple, rather than through the air or solution, since the thermocouple is the path of highest thermal conductance and since the 25-30 msec delay occurs both in air and in solution.

Davis (28) has outlined the following criteria for valid temperature measurements in in vitro biological systems based on the fundamental condition that temperature is defined only when a system is in thermal equilibrium (no thermal energy exchange with environment or other parts of the system). A thermometer is introduced as an auxiliary thermodynamic system when the biological system in question exhibits no useful thermometric characteristic of its own. The thermometer must not perturb the thermodynamic state of the system whose temperature is to be measured. The measurement is only valid only when the system and thermometer achieve and maintain thermal equilibrium (no thermal energy exchange between thermometer and system). Thermometers with low heat capacity and high heat transfer coefficient relative to the system can follow the temperature excursions of the system closely but never exactly. In those cases where both the system and thermometer are in contact during a heating period, the thermometer and system must respond identically for a valid temperature to be registered. In the case where an opaque thermometer is immersed in a translucent biological medium and the two systems are heated together by a radiant energy input thermal equilibrium is almost impossible to maintain between the thermocouple and biological system. In a biological system where transient heat flow occurs, temperature is undefined except within an infinitesimal volume where no temperature variation is assumed to occur within this volume. If arbitrarily small volume elements are assumed to lie in equilibrium temperature states, then a small thermometer can measure the temperature of a volume without experiencing a temperature gradient across the sensing element (27).

B. Modes of Mechanical Interaction

At low energy density and power density levels, the gross interactions of laser radiation with biological systems are due mainly to temperature elevation *per se*. As the power and energy density is increased, mechanical interactions due to phase transformations, tissue volatilization, thermal expansion, and quantum processes involving phonons (quanta of vibrational energy) become important. The effects of mechanical interactions may exhibit characteristics different from the effects of temperature elevation *per se*.

Burkhalter (1) and Fine *et al* (5) have investigated the mechanical effects of laser interaction with torsion and ballistic pendulums. Fine *et al* have reported that when the blackened surface of a ballistic pendulum was exposed to a laser pulse, oscillations were observed. This occurred when the pendulum was mounted in air or in a vacuum. Oscillations were not observed, however, when the unblackened surface was exposed (15). These oscillations appear to be caused by conservation of momentum at the pendulum surface, rather than by radiation pressure, thermal gradients, or heating of the air at the surface (1,5). Burkhalter has explained that the recoil impulse imparted to a torsion pendulum by volatilized mass blown away from at the pendulum surface can be determined by measuring the torsion constant of the pendulum system, the radius at which the impulse is applied, the initial angular displacement of the system, and the period of damped oscillation following the impulse (1). Numerical data associated with this recoil effect has not yet been published by Burkhalter and would be desirable. At the surface of a

biological system, it is possible to impart a net momentum change to the biological target via tissue volatilization (excluding the volatilized material itself). At higher energy and power density levels, volatilization and phase changes may occur deep to the surface, whereby a net momentum change is not imparted to the biological target as a whole, yet strong pressure transients may be generated within the system. The mechanism of surface volatilization is probably not of major importance in damage deep to the surface at high energy and power levels (29)

Fine et al (5) have investigated pressure transients generated by phase changes occurring deep to the surface of biological systems. A model for steam production has been adopted where quasi-static conditions (constant pressure throughout the volume of water vapor) are assumed to exist during a phase change induced by a ruby laser pulse lasting on the order of one millisecond. Radiation from Q-switched systems can produce a relatively rapid pressure increase in comparison with radiation from comparable non-Q-switched systems. With Q-switched systems, a rapid pressure build-up can occur which may result in the transmission of a shock wave. These shock waves may travel with relatively little attenuation and consequently affect areas at some distance from their origin (5).

This model has been applied to situations where outward, hemispherical distensions of the abdominal wall of mice were observed (5) when non-Q-switched radiation at 6943 Å at energy levels in the 2 - 20 joule range was directed at the abdominal surface. High speed photography has indicated that the millisecond duration of the distension was of the order of the laser pulse duration. Assuming that nearly static pressure equilibrium

is established in the tissue over an interval of 10^{-4} sec. (based on the velocity of sound in water vapor at 100°C), it is shown that, for a distension radius as large as 1 cm, a pressure exceeding 1 atmosphere may be obtained at temperatures a little over 100°C. There is , of course, an upper limit to the pressures that will be generated before the containing membranes burst. This limit depends on the stratum in which the vapor is being collectdd, and on the nature of the overlying tissue (especially its burst strength, which may be dependent on strain rate) (5).

Pressure transients may be created by thermal expansion without change of phase, In studies on physical systems, the generation of elastic waves have accompanied the absorption of radiation from high power light sources (including electric arcs and ruby lasers), microwave sources, and the absorption of pulsed electron beams within solid targets. Radiation absorption may produce local temperature gradients and, as a result of thermal expansion, cause stress gradients within the target medium. Pressure waves then propagate from the stress concentration and may create shock fronts within the target (29).

In biological systems, thermal gradients (and hence stress concentrations) will appear at the target surface or internally during the absorption of laser radiation. Where refractive index changes occur at interfaces within biological systems, enhancement of radiation absorption can exist, due to multiple reflections, therefore giving rise to stress gradients beneath the surface (5). Pressure waves may then propagate through the biological systems with possible shock fronts at the leading and trailing edges of the wave train.

Absorption of laser energy within a closed, filled cavity, such as the eye or skull, differs from that occurring at a free surface or within a cavity with non-rigid walls (31). Phase changes that occur within filled cavities with rigid walls will generate high pressures within the cavity when quasi-static conditions can be assumed to hold. The importance of cavity boundaries can be determined by considerations of sound velocities within the cavity and laser pulse duration. If pressure transients, travelling at the speed of sound, interact with rigid cavity walls during a phase transformation then greater pressures will occur than if the cavity walls were flexible. Under quasi-static conditions, pressure pulses can be transmitted with relatively little attenuation to regions distant from the site of interaction. This can result in tissue destruction due to direct mechanical effects and/or due to disruption of vascular supply at some distance from the site of original laser impact. Consequently, severe and sometimes fatal injuries can be produced, although the local effects at the site of original laser impact may not be vital to the functioning or survival of the organism.

Experimental studies (11) Amar) have indicated the importance of laser interaction within closed filled cavities. Fine and Klein (31,5) have reported studies of the effects of pulsed unfocused and focused irradiation of the forehead in mice and have discussed the importance of the intact skull in these studies. The hair on the forehead was clipped and the area depilated. Of 41 animals irradiated at energy levels below 100 joules, 31 died within 24 hours of irradiation. Unfocused irradiation was followed by death within 30 seconds in 10 out of 23 animals. Neurological changes were noted in the animals which survived irradiation (31).

Hemorrhages from the mouth, nose, auditory meatus, retro-orbital space and orbit were observed. Although subperiosteal petechiae were present on the outer table of the cranial bones, gross damage to the underlying bone was not evident. Gross intracranial hemorrhages were observed in the meningeal space, the ventricles, the conducting system and within the substance of the brain distant from the site of impact. Microscopically, hemorrhages were seen in the underlying muscle in addition to cutaneous lesions. The bone marrow showed elongated and condensed nuclei on microscopic examination. Hemorrhages were present in the meninges and meningeal spaces, as well as within the cerebral and cerebellar cortex, within the ventricles and at the base of the brain. Sharp demarcation of intracranial lesions was not observed grossly or microscopically (31).

At energy levels exceeding 100 joules per pulse, similar results were obtained on irradiation directed at the forehead. However, at energy levels in excess of 500 joules, rupture of the skin overlying the skull with folding back of the edges at the site of the rupture was observed. Preliminary studies indicated that, in the 50 to 100 joule range, at 6943 $\mathring{A}$, incident radiation of the order of 10 percent directly penetrates through the skin, muscle, and skull. In control studies, the exposed brain was directly irradiated at energy levels of 10 to 12 joules. Lesions produced in these animals were localized to the sites of irradiation and were compatible with survival (31). These studies are indicative of the importance of laser interaction within a closed, filled cavity such as the cranium.

Studies were carried out by Earle et al (32) on the effects of radiation at 20 joules per pulse at 6943 Å directed at the unshaven forehead of mice and rats. With unfocused radiation, the hair and scalp of white mice were burned, but no immediate or late effects were found in the brain. With the beam focused to an area 2 mm in diameter on the scalp, a deep burn was produced in the skin, the cranium was intact, and acute subdural, subarachnoid, and slit-like intracerebral hemorrhages were produced in the brains of some mice, but not of rats. With the beam focused so that the focal point would be within the brain, if transmitted through scalp and cranium, acute ischemic necrosis and slit-like hemorrhages were produced in brains of rats and mice. The effects were fatal to mice within a few minutes. The rats appeared to be dazed, but were not killed. Two rats were allowed to live for eleven days, and the late effects were found to show features of healing contusions of the brain (32).

In subsequent studies by Earle and Hayes (33), the unshaven heads of unanesthetized white mice were exposed to unfocused and focused (into the brain) laser radiation at 30-35 joules per pulse at 6943 Å. Thirty-seven animals following unfocused irradiation did not show ill effects, whereas all of the 16 animals exposed to a beam focused 2 mm into the brain showed immediate severe neurologic symptoms, which culminated in death. These results were considered predictable by a thermal hypothesis, since 100 joules/cm^2 incident on the skin of the head results in 10 joules/cm^2 incident on the brain. If the absorption coefficient is 16 cm^{-1} at 6943 Å and the specific heat 1 cal/gm°C, the outer 100 microns was computed to show a temperature increment of 46°C. If this energy were focused to 1 mm^2, however, the predicted thermal effect would be

increased a hundredfold, and severe damage and death would occur (33).

The brains of anesthetized cats were exposed through the dura to graded energy fluxes from a ruby laser. A 45° cone angle was utilized in an attempt to produce a trackless deep lesion. Immediately after exposure, the animals were injected with trypan blue, and, after one hour, perfused. Graded effects (intradural and subdural hemorrhage) and increased permeability, as evidenced by the dye, were observed. All of the lesions appeared to be in continuity. Deep lesions with sparing of superficial tissues were not observed at these energy and power density levels. While the studies by Earle et al. and Earle and Hayes are of interest, further experimental evidence is necessary to separate the effects of closed cranial cavity from thermal effects per se.

A possible model for the above, should include the possible refractive or reflective effects of skin, bone, and brain upon the position of the focal point of a focused laser beam that penetrates the cranium of a mouse. The focal point in air can be expected to shift as much as 30% and experience certain aberrations (neglecting radiation scatter) when the intact mouse skull is moved into the path of the laser beam. Factors such as refractive indices, skull curvature and the external optics (especially beam convergence or divergence angle) used must be considered.

The following model considered by the reviewers may enable an initial temperature rise to be determined near the beam edge at the brain surface. For a converging beam in tissue, the temperature rise near the beam edge at the tissue surface is higher than the temperature near

the beam axis at the tissue surface. Assume that the focal depth of 2 mm into the beam. is correct when head curvature, refractive indices of the head, and beam convergence angle in air are considered. Assume the absorption coefficient of 16 cm^{-1} suggested by Earle and Hayes for the mouse brain, an incident energy density at the brain of 10 joules/cm^2 (suggested by Earle and Hayes (33)), and a beam cross section of 1 cm^2 at the brain surface (suggested by Earle and Hayes (33)). The initial surface temperature rise near the beam edge is given by:

$$T \approx \frac{\text{(Energy density) (Absorption Coeff) (sec 68°)}}{\text{(4.18 joules/cal.)}}$$

$$T \approx 100^{\circ} C$$

The temperature rise near the center of the beam is approximately 40°C. If normal ambient temperature (approximately 35°C) is added to these figures, it can be seen that around the periphery of the irradiated surface of the brain, temperatures will exceed the steam point. One can estimate that due to possible phase changes within the mouse cranium, the biological effects observed (33) may not be thermal per se.

Experimental studies have been reported by Fine et al (30) where pressure transducers were inserted through small openings in the otherwise intact skull of mice, and pressure transients were measured on laser irradiation of the forehead. A much higher pressure was recorded within the closed, filled cranium, then when a section of skull and skin were irradiated at the same energy level with the pressure transducer placed

behind and in contact with the skull (Figure 3) (30).

The presence of sonic pressure waves during the interaction of laser radiation with both *in vivo* and *in vitro* biological systems has been reported by Fine *et al* (34). In further studies (30), both sonic and ultrasonic, incoherent pressure waves have been detected during non-Q-switched (approx. 20 joules) and Q-switched (approx. 1 joule) ruby laser irradiation of heads and chests of mice. These frequencies were detected when the anterior surfaces of the chest and head were irradiated with pressure transducers coupled via a water film to the posterior surfaces of mice (30).

Amar *et al* (35) have reported the production of pressure transients in the occipital bone of rabbits, when the interior of the eye was irradiated through the pupil with a fifty milli-joule, 400 μsec pulsed laser. Radiation directed at the sclera, or iris, resulted in a factor of ten decrease in pressure amplitude at the occipital bone. No pressure response was noted for the same radiation level directed at other portions of the rabbit face (for example, the cartilage of the nose) (35). These results apparently confirm the importance of laser interaction within closed cavities with rigid walls. The pressure transients were recorded with a barium titanate crystal transducer affixed to the occipital bone. The principle component of the pressure response was reported to be approximately 40,000 c.p.s. Piezoelectric crystals in conjunction with monitoring circuitry can be reduced to an equivalent R-L-C electric circuit. Resonance in R-L-C oscillating circuits enhance power output for certain frequencies. For this reason it would have been desirable for Amar *et al* to include the

frequency response for their transducer system in order to ascertain whether the 40 Kc. component was characteristic of the transducer system of the laser interaction (35).

Marchal et al (36) have irradiated excised cerebral tissue, which was compressed within a rigid plexiglass container. The laser used was a pulsed ruby operating at 4 joules. The tissue was irradiated through an open end of the plexiglass container, and ultrasonic pressure waves were detected in the 250 kilocycle region by a barium titanate crystal coupled to the oposite closed end of the container. The crystal was rated at 15 megacycles. However, it is not clear whether this response was related to the specific monitoring equipment used in the experiments described. The transducer oscilloscope traces shown in the article contain an extremely high D.C. component (approx. 10 times the oscillatory signal), which indicates possible heating of the crystal by scattered or direct laser light. However, the cause of this D.C. component was not discussed by Marchal.

Other experiments are described in which a plexiglass plug covered with aluminum foil was placed over the same plexiglass container filled with water. The laser was focused within the plug, and ultrasonic pulses were again detected at the bottom surface of the container. The signal was 10 percent of that obtained when the plug was removed (36). No indication was given as to the relative amplitudes of the D.C. and A.C. levels for this set of experiments. Such data would have been helpful s since a 10 percent reduction in signal could have been effected by eliminating only the D.C. component. Elimination of the D.C. component may have been indicative of transducer heating when the aluminum foil shield was not used.

The crystal transducer was removed and replaced by a photodiode resulting in no diode signal when some or all of the above experiments were repeated. It is not clear from the article, however, which experiments were repeated with the photodiode in place. Further data using the photodiode may have cleared up the problem of transducer heating.

Studies on pressure wave formation and transmission have been carried out by Mendleson et al using various physical models (37). Pressure waves, primarily of acoustic velocity, occurred within a gelatin model following irradiation with a Q-switched laser at 3 joules (0.5 joules per pulse). The pressure waves were rapidly attenuated with depth in the gelatin (37).

Attempts at accurate, direct reading of pressure amplitudes have met with difficulties in the calibration of pressure transducers. While static calibration of small piezoelectric crystal transducer (e.g. barium titanate) is feasible, dynamic calibration for a wide range of frequencies is difficult to achieve. Since the electrical and mechanical characteristics of piezoelectric transducers are not independent, monitoring circuitry must be carefully designed to match both purely electrical and electrically equivalent mechanical characteristics of the crystal transducer. The transducer must be calibrated in conjunction with the monitoring system to be used during the course of the experiments and recalibrated if a new monitoring system is employed.

Careful planning of experiments can allow relative measurements to be made where only one variable of an uncalibrated transducer system output is allowed to change (e.g. frequency or amplitude but not both). Irradi-

ation of the transducer itself, by either direct or scattered light, must be carefully nulled in comparative studies with an uncalibrated system.

Bubble production in liquids during both Q-switched and non-Q-switched laser irradiation has been reported (38,39,40). Weiss (38) has detected bubble production during studies on laser activated photochemical reactions. It has been suggested that foreign material, such as dust particles, may create centers for local heating within the chemical system. Absorption of laser radiation by dust particles may then lead to bubble formation at localized sites. Another possibility is the presence of gas in the system.

Desvignes et al (39) have photographed bubbles that have formed in human vitreous and persisted for several hours following pulsed ruby laser irradiation of the isolated vitreous. Four joule laser pulses of approximately 500 μsec duration were focused near the free surface of human vitreous held in plexiglass containers. Gross absorption of laser radiation in the vitreous was too low to directly cause change of phase, yet bubbles were observed in the path of the beam. Desvignes has suggested that a correlation exists between the ultrasonic waves detected by barium titanate transducers located at the bottom, outer surface of the plexiglass container and bubble formation in the beam path. Ultrasonic cavitation is suggested as the mechanism for bubble production.

Fine et al (40) have detected bubbles formed in water during Q-switched studies on model systems with reflecting interfaces. High speed framing photographs from an STL image converter camera showed transient bubble production along the beam path of a Q-switched laser focused at the surface of a metal plate submerged in a glass container of water. In other unpublished studies by Fine et al, bubbles have been detected in gelatin blocks, particularly at interfaces.

Chiao, Townes, and Stoicheff (41), have shown that irradiation of certain crystals by a 50 megawatt, 30 μsec, ruby laser pulse results in the production of intense (1 kilowatt), coherent, hypersonic waves (10^{10} c.p.s.) via stimulated Brillouin scattering. Studies carried out by Garmire and Townes (42) have shown that, in a similar way, intense, coherent, hypersonic waves can be generated in liquids such as water. Giulano (43) has investigated damage in dielectric solids caused by hypersonic waves (13 g.c.), which were created via stimulated phonon processes, both within the solid material and within the liquids surrounding the solid. Gigacycle waves are rapidly attenuated, thus giving rise to possible damage only in regions near the laser beam itself. Should coherent hypersonic waves be generated in biological systems, these waves may result in cell death, or alteration along the laser beam path.

REFERENCES

1. Burkhalter, J.H., Federation Proc. Suppl. 14, 24 (1), Pt. III, S-31, 1965.
2. Yura, H.T., "The Interaction of Laser Light with Metals", Memorandum RM-3560-PR, Mar. 1963, AD 401 478.
3. Fairbanks, R.H., and Daly, R.T., "Laser Beam Fusion Welding" T.R.G., Inc., Oct. 1962, ASTIA, AD 291 815.
4. Schineller, E.R., et al., Wheeler Laboratories Report 1182A, Dec. 1963.
5. Fine, S., et al., "Interaction of Laser Radiation with Biologic Systems, I., Federation Proc., Suppl. 14, 24 (1), Pt. III:S-35, 1965.
6. Hansen, W.P., Fine, S., et al., to be presented at 1965 NEREM.
7. Helwig, E.B., et al., Federation Proc., Suppl. 14, 24(1), Pt. III, S-83, 1965.
8. Hardy, J.D., et al., J. Appl. Physiol. 9:257, 1936.
9. Davis, T.P., "The Heating of Skin by Radiant Energy", in C.M. Herzfeld, Temperature, Its Measurement and Control in Science and Industry, (New York: Reinhold, 1963) Vol 3:149.
10. Henriques, F.C., Jr., and Moritz, A.R., Am. J. Pathol. 23:531, 1947.

11. Kuppenheim, H.F., et al, J. Appl. Physiol., 9:75, 1956.

12. Ham, W.T., Jr., et al, "Ocular Effects of Laser Radiation, Part I," DASA - 1574.

13. Vos, J.J., "A Theory of Retinal Burns," Bull. Math. Biophys., 24:115, 1962.

14. Ham, W. T., et al, Amer. J. Ophthal. 46:700, 1958.

15. Davies, J.M., "The Effect of Intense Thermal Radiation on Animal Skin, A Comparison of Calculated and Observed Burns; Report T-24, Quartermaster Res. and Engineering Center, Natick, Mass., 1959.

16. Felstead, E.B., and Cobbold, R.S.C., "Analog Solution of Laser Retinal Coagulation, Med. Electron. Biol. Engng., 2:145, 1965.

17. Campbell, Charles, J., et al, "Intraocular Temperature Changes Produced by Laser Coagulation," Acta Ophthal. Suppl. 76:22, 1963.

18. Noyori, K.S., et al, "Ocular Thermal Effects Produced by Photocoagulation," Arch. Ophth. 70:119, 1963.

19. Campbell, C.J., Rittler, M.C., and Koester, C.J., "The Optical Maser as a Retinal Coagulator: An evaluation," Trans. Amer. Acad. Ophthal. and Otolaryngol, 67:58, Jan.-Feb. 1963.

20. Nowak, W.B., et al, "On the Use of Thermocouples for Temperature Measurement During Laser Irradiation, Life Sciences, 3 (11):1475, 1964.

21. Geeraets, Walter J., and Ridgeway, D., "Retinal Damage from High Intensity Light," Acta Ophthal. Suppl. 76, 1963.

22. Geeraets, W.J., et al, "The Relative Absorption of Thermal Energy in Retina and Choroid," Invest. Ophthal. 1:340, 1962.

23. Najac, H. et al, "Direct Thermocouple Measurements of Temperature Rise and Heat conduction in the Rabbit Retina," Invest. Ophthal. 2:32, Feb. 1963.

24. Crowder, J., "Measurements of the Vitreous Temperature During Photo-Coagulation in the Rabbit Eye,"Acta. Ophthal. Suppl. 76:32, 1963.

25. McCartney, A.J., and Hall, W.F., "Temperature Measurements in a Mouse Tumor System Following Ruby Laser Exposures," presented at First Annual Biomedical Laser Conference of the Laser Medical Res. Foundation, June 17-18, 1965.

26. Masket, A.V., "Time Reversal in Heat Conduction," Amer. J. Phys. 33:196, March 1965.

27. Data Sheets, Micro-minature thermocouple, type TCA-ET-200, Baldwin-Lima-Hamilton Company, W ltham, Massachusetts.

28. Davis, T.P., "In Vitro Temperature Measurements," Acta Opthhal. Suppl., 76:41, 1963.

29. Fine, S., and Klein, E., "Biological Effects of Laser Radiation," Advances in Biological and Medical Physics, (in press).

30. Fine, S., et al, Mechanisms and Control of Laser Hazards and Management of Accidents," presented at the second conference on Laser Technology, held at the Illinois Institute of Technology, Chicago, Ill., 6-8 April, 1965. (Published in Conf. Proceedings)

31. Fine, S., Klein, E., "Effects of Pulsed Laser Irradiation of the Forehead in Mice," Life Sciences, 3(3):199, 1964.

32. Earle, et al, "Central Nervous System Effects of Laser Radiation Federation Proc., 24, (1), Part III, Suppl 14, Jan-Feb 1965.

33. Earle and Hayes, presented at the 3rd Annual Boston Laser Conf., Aug 1964.

34. Fine, S., Klein, E., Laor,Y. "Modification of Effects of Laser Radiation by Light Absorbing Chemicals," presented at Third Boston Laser Conference, 1964.

35. Amar, Leo, et al, "Detection d'ondes elastiques (ultrasonores) sur l'os occipital, induites par impulsions laser dans l'oeil d'un lapin," C.R. Acad. Sc. Paris, 259:3653 (13), Nov. 1964.

36. Marchal, Maurice et al, "Sur la detection d'ondes ultrasonores induites dans le tissu cerebral par irradiation d'impulsions Laser," C.R. Acad Sc. Paris, 259 (13): 15 July 1964.

37. Mendelson, Janic A., and Ackerman, N.B., "Studies of Biologically Significant forces Following Laser Irradiation," FederationProc. Suppl 14 24 (1), Pt III, 1965.

38. Weiss, K., et al, "Laser Applications in Photochemistry," presented at Third Boston Laser Conference, Aug. 1964.

39. Desvignes, Pierre, et al, "Sur la generation d'ondes ultrasonores et formation de bulles dans le vitre d'un oeil humain par irradiation d'impulsions laser, C.R. Acad. Sc. Paris, 259 (13): 24 Aout 1964.

40. Fine, S. et al, "Measurements and Hazards on Interaction of Laser Radiation and Biological Systems," NEREM Record 1964.

41. Chiao, R.Y., Townes, C.H., Stoicheff, B.P.: "Stimulated Brillouin Scattering and Coherent Generation of Intense Hypersonic Waves," Phys. Rev. Letters, 12 (12), May, 1964.

42. Garmire, E., Townes, C.H., "Stimulated Brillouin Scattering in Liquids," Phys. Rev. Letters, 5 (5), Aug. 1964.

43. Giuliano, C.R., "Laser-induced Damage to Transparent Dielectric Materials," Appl. Phys. Letters, 5 (7), Oct. 1964

Radiometric and Photometric Units

A specific portion of the electromagnetic spectrum, called the visible region, consists of radiation which stimulates the sense of vision. In the limited wavelength interval from 3800 Å to 7600 Å, a system of units, the photometric system, is used to describe the average response of the eye. There is another unit system, the radiometric system, which describes the physical nature of radiation at any wavelength but does not supply any information with regard to the physiological aspect if vision. In biophysical studies, particularly with respect to ophthalmology, a problem arises concerning which system of units, photometric or radiometric, to use. The most important factor to be considered is, of course, which system will more accurately describe the particular situation. In studies concerned with the physiology of vision the photometric system is a convenient choice. With reversible impairment such as dazzle and glare, this system of units is not applicable. If the object of the study is the mechanism or thresholds of irreversible damage, such as retinal burns, or reversible injury, such as keratitis or keratoconjunctivitis, then a description of the radiation and effects in terms of radiometric units may be more useful. It is the purpose of this section to discuss both unit systems, with emphasis on the photometric, and to justify the above statements.

Photometry and the photometric unit system have been used for many years in illumination engineering and vision research (1,2,5,7). Typically such units as lumens, candles, trolands, lux and lamberts are used. The basic area of interest of photometry includes the measurement and computation of the emission, propagation, absorption, and reflection of light in the visible region, that is 3800-7600 Å. Although visual response has

been measured to the wavelength region of 9000 Å for sufficiently intense stimulation (1) it is not considered in the usual definitions of Photometry. Photometry is concerned with, and photometric units are based on, the quantitative evaluation of radiation in the 3800-7600 Å interval with respect to its capacity to stimulate visual sensation. The basis of photometry is the sense of vision and the units of photometry are based on the response of the human eye in the above wavelength region only.

In order that photometry not be fully subjective, since visual sensitivity varies from person to person, a standard visual sensitivity curve, called the luminosity curve was adopted by the International Commission on Illumination in 1924 (2). For standardization purposes the average photopic, or light adapted eye, sensitivity curve K_λ is used as the basis for all photometric units (2). The photopic as well as the average dark - adapted, or scotopic V' response curve is shown in Figure I. The scotopic curve in Figure I illustrates the Purkinje effect, which is the shift in wavelength of the sensitivity of the eye as a function of illumination. Photometric units are defined in terms of the photopic eye and visual photometric measurements of brightness cannot be uniquely related to the photometric illumination except in the photopic region (2). All photometric units, thus, are referred to units defined in terms of the light - adapted eye and its response and are not related to scotopic vision. This is no great stumbling block to photometric measurement, but it can be a source of error if visual photometry is carried out under various illumination conditions.

As long as measurements using photometric units are consistent with the definition of the units no difficulties arise. However, evaluation of physical phenomena on the basis of the photometric unit system relates all measurements to the subjective quality of vision. No photometric measure-

ment can describe the physical interaction of optical radiation with matter. Radiation sources are specified in terms of their visual output. Illumination of targets or receivers are described in terms of how visible or how bright they appear. Only by a radiometric description of the radiation source in terms of units such as watts, joules, or ergs, can a description be given as to how much power or energy is emitted or how much power or energy is incident on a target. Furthermore, only by a description in radiometric units can the power at different wavelengths be given. Only in the radiometric system can radiation of wavelength shorter than 3800A or longer than 7600A be described at all. The comparison of units (Table I), and their meanings more fully shows the general nature of the radiometric system. The notation used is that recommended by the Optical Society of America. (4).

The completeness of radiometric units can be seen from this table as well as the major fault of the photometric units. There are no photometric units which involve the spectral distribution of radiation.

The two basic units of photometry are defined in the following manner:

The candela, formerly the candle, the basic unit of luminous intensity, is defined as the luminous intensity of one-sixtieth square centimeter of a blackbody at the melting temperature of platinum (2040°K) (3).

The lumen, the basic unit of luminous flux or power is defined in terms of the radiation source. The luminous output, F, in lumens, of a light source of a spectral power distribution, P_λ is defined as (2):

$$F = 680 \int_{\lambda_1}^{\lambda_2} P_\lambda \, K_\lambda \, d\lambda \quad \text{lumens} \qquad (1)$$

where K_λ is the normalized photopic luminosity function, shown in Figure I,

TABLE I

A Comparison of Radiometric and Photometric Units

Radiometric			Photometric		
Symbol	Name	Unit	Symbol	Name	
U	Radiant Energy	joule	Q	Luminous Energy	talbot(lumen-second)
u	Radiant Energy Density	joule/cm^3	q	Luminous Energy Density	talbot/m^3
P	Radiant Power (Flux)	watt	F	Luminous Flux(Power)	lumen
W	Radiant Emittance	watt/cm^2	L	Luminous Emittance	lumen/m^2
H	Irradiance	watt/cm^2	E	Illuminance	lumen/m^2(lux)(meter-candle)
J	Radiant Intensity	watt/sr	I	Luminous Intensity	lumen/sr (candela)
N	Radiance	watt/sr-cm^2	B	Luminance	lumen/sr-m^2(candela/m^2)
P_λ	Spectral radiant Power	watt/micron			
W_λ	Spectral Radiant Emittance	watt/cm^2-micron			
H_λ	Spectral Irradiance	watt/cm^2-micron			
J_λ	Spectral Radiant Intensity	watt/sr-micron			
N_λ	Spectral Radiance	watt/sr-cm^2-micron			

where cm= centimeter, m=meter, sr=steradian, micron is a unit of wavelength equal to 10^{-6} meters. It is also common for spectral units to be given per 0.001 micron (1 nano-meter or millimicron) or per 0.01 micron (10 millimicrons or 100 Angstroms).

Note: 1 Angstrom(A) = 10^{-8} cm = 10^{-10} meter

1 millimicron = 10A = 10^{-7}cm = 10^{-9} meter

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and the limits of integration extend over the visible region. Table II gives conversion factors for commonly used units of luminance and illumination.

The unit of retinal illumination, the troland, is included in the units of illumination since it is a unit of physiological studies (12). The troland or luxon is defined as the retinal illumination per square millimeter of pupil area produced by a surface having a luminance of one candela per square meter (3). Due to the Stiles-Crawford effect, the fact that light entering near the edge of the pupil is much less effective visually than rays near the center of the pupil, for pupil diameters of about 2.5 millimeters or greater; the troland cannot generally be used for pupils larger than 2.5 millimeters. However, physiological measurements can be made using artificial pupils which limit the area of the natural pupil through which light enters or an equivalent natural pupil size can be calculated as pointed out by Moon and Spencer (13).

It is important to note that the troland only indicates the total luminous flux entering the eyes. From its definition in terms of the luminance of a surface, it would seem to imply that the retina is illuminated over the full spherical surface subtended by the same interior solid angle which the illuminating field subtends exterior to the pupil, as shown in Figure II. This fact is pointed out in Duke-Elder (14). Thus the use of the troland as a unit of retinal illumination implies nothing concerning the luminous or radiant power density of the retinal surface unless the illuminating field and pupillary diameter are specified simultaneously.

The units of the radiometric system are defined in terms of the three basic units of physics - length, mass, and time (L,M,T). For example:

1 joule = 1 newton

= 1 (kilogram-meter/sec^2)-meter

TABLE II

Definitions of Various Photometric Terms

Illumination Units (Flux per unit area)

1 lux (meter-candle)	=	1 lumen/meter^2
1 Phot	=	1 lumen/cm^2
1 foot-candle	=	1 lumen/foot^2
1 troland (luxon)	=	$\frac{1}{\pi}$ lumens/meter^2 (for a lambertion diffusing surface)

Luminance Units
(Intensity per unit area or flux per steradian per unit area)

1 nit	=	1 lumen/sr/meter^2 = 1 candela/meter^2
1 stilb	=	1 lumen/sr/cm^2 = 1 candela/meter^2
1 candela (candle)ft^2	=	1 lumen/foot^2
1 apostilb	=	$\frac{1}{\pi}$ nit
1 lambert	=	$\frac{1}{\pi}$ stilb
1 foot-lambert	=	$\frac{1}{\pi}$ candela/ft^2

Abbreviations used:

sr = steradian

cm = centimeter

TABLE III

Luminance Conversion Factors

	Stilbs	Candles/in^2	Apostilbs	Lamberts	Ft.-Lamberts
1 nit	10^{-4}	$.6452 \times 10^{-3}$	3.142	$.3142 \times 10^{-3}$	.2919
1 stilb	1	6.452	31,420	3,142	2919
1 candle/in^2	.155	1	4869	.4869	452.4
1 Apostilb	31.83×10^{-6}	$.2054 \times 10^{-3}$	1	10^{-4}	.0929
1 Lambert	.3183	2.054	10^4	1	929
1 milli-Lambert	$.3183 \times 10^{-3}$	2.054×10^{-3}	10	10^{-3}	.929
1 foot-Lambert	$.3426 \times 10^{-3}$	2.21×10^{-3}	10.76	1.076×10^{-3}	1

Illumination Conversion Factors

	Foot-candles	Luxes	Phots
1 Foot-candle	1	10.76	1.076×10^{-3}
1 Lux	.0929	1	10^{-4}
1 Phot	929	10^4	1
1 Troland	.292	3.142	3.142×10^{-4}

or, dimensionally, one joule has the dimensions ($ML^2 T^{-2}$) and since one watt equals one joule/second the dimensions of the watt are (ML^2T^{-3}).

There are three basic systems of physical units on which radiometric units can be based. The two metric systems are the MKS and the CGS, and the third system is the British. The MKS system is the most commonly used system, although the CGS is sometimes used. The definition of some radiometric quantities and conversion factors are given in Table IV. Included also are the thermal units of energy, the calorie and the B.T.U., which are occasionally used as radiometric units.

Calculations using the units of either system, within that system, is straightforward once the unit of power (flux) is known. For example, a radiation source emitting one watt of power, P, will, in one second, radiate a total of one joule, U, of energy. A source with a luminous flux (power) of one lumen will, in one second, radiate a luminous energy of one talbot. For a source that emits radiation isotropically the radiant or photometric power (flux) density will follow the inverse square law, that is, at a distance R (centimeters, or meters) from a source of output P (or F) the power density emittance will be:

$$W = P/4\pi R^2 \quad \text{watts/cm}^2 \quad \text{or} \quad L = F/4\pi R^2 \quad \text{lumens/m}^2$$

The intensity of the same source will be:

$$J = WR^2 = P/4\pi \qquad \text{or} \qquad I = LR^2 = F/4\pi$$

It can be seen from the description of the two unit systems that the radiometric system is fundamental, that is, they are based on the common units of physics and chemistry. On the other hand, the photometric units are tailored for relative descriptions of visual sensation.

TABLE IV

Definition of Physical Units of Energy and Power

Energy

MKS	1 joule = 1 newton-meter
CGS - physical	1 erg = 1 dyne-centimeter
- thermal	1 calorie = amount of heat energy required to raise the temperature of one gram of water from 14.5°C to 15.5°C
British - physical	1 foot-pound = 1 foot-pound
- thermal	1 B.T.U. = amount of heat energy required to raise the temperature of one standard pound of water at 63°F by one degree Fahrenheit

Power

MKS	1 watt = 1 joule/second
CGS	1 erg/sec = 1 erg/sec
British	1 ft-lb/sec = 1 foot-lb/sec

Conversion Factor

	joule	erg	calorie	ft-lb	B.T.U.
1 joule =	1.0	10^{-7}	.2389	.7376	9.481×10^{-4}
1 erg =	10^{-7}	1.0	2.389×10^{-8}	7.376×10^{-8}	9.481×10^{-11}
1 calorie =	4.186	4.186×10^{7}	1.0	3.087	3.968×10^{-3}
1 ft-lb =	1.356	1.356×10^{7}	.3239	1.0	1.285×10^{-3}
1 B.T.U. =	1055.0	1.055×10^{-10}	252.0	777.9	1.0

In order that the luminous output of a light source be specified, its radiation distribution must be known in radiometric units, watts per micron. Thus a radiometric description of radiant processes can be converted to a luminous description by use of equation one. Photometric descriptions, on the other hand, cannot generally be converted to radiometric ones since a given luminous unit is a number and not indicative of the physical power involved or its distribution in wavelength. In other words, a large variety of radiation source of varied spectral radiant power can be combined in equation one, to give the same value of luminous flux. Unless the P_λ of the source is stated separately one cannot obtain a radiometric description of events. In the case where the luminous output of a source is given, as well as the spectral power distribution, physical data can be interpreted directly in terms of the P_λ information. Table V compares several light sources, including single wavelength lasers, under the requirement that each source have the same luminous output. It can readily be seen that, especially in the case of the lasers, the specification of the luminous output does not characterize the power output.

Since most lasers operate at one wavelength, the K_λ is found from the luminosity curve and multiplied by 680 times the laser power output to determine the luminous flux in lumens. The inverse process works equally well, since only one wavelength is involved and the integral of equation one is replaced by the simple equation

$$F_{laser} = 680\ K_\lambda\ P_\lambda \text{ lumens}$$

In the assessment of ophthomological hazards with respect to laser radiation, photometric units are not only inadequate, since some infrared

TABLE V

Total Power Output of Several Light Sources of Different Spectral Distribution

Required to Produce a Luminous Output of 1500 Lumens

Source	Spectral Power Distribution	Total Radiometric Power Output
Incandescent Lamp	Approx. Blackbody Distribution*	9-10 watts
Fluorescent Lamp	Phosphor Fluorescence Distribution*	4-5 watts
Helium-Neon Laser	Single Wavelength λ =6328	88 watts
Argon Laser	Single Wavelength λ =5145	4 watts
GaAs Laser	Single λ =8900	Undefined (very high)
Argon Laser	λ =3002	

* See reference 1

reaches the retina (10,11), but they are misleading since 90% of the radiation between 4000 Å and 9000 Å are transmitted by the refractive media of the eye (11). Yet in this latter wavelength interval the K_λ, luminosity curve, varies from its full maximum to zero. Accordingly, any attempt to classify laser hazards in terms of conventionally defined photometric units is meaningless unless a hazard level is specified at each wavelength considered. This is a most arduous task and it would be far easier to determine hazard levels in terms of radiant power or energy levels. Using radiometric units it may be possible to define ophthomological hazards with one or two specifications regarding wavelength.

In the section on Gas Lasers, it was shown that using a surface energy density of 1 joule/cm^2 at the retina as a damage threshold value, it may be possible to create a retinal lesion with a one milliwatt laser beam at 6328 Å in one millisecond. It seems reasonable to assume this same threshold value for any wavelength in the interval of 4000 to 9000 Å based on the uniform transmission of the ocular media (11). This hypothesis seems very reasonable and is commented on explicitly by Duke-Elder (11). The exact words used are "Such lesions are therefore caused indiscriminately by infrared or visible light, the reaction being unrelated either quantitatively or qualitatively to any particular wavelength but depending on the concentration of energy incident in this region".

In Table VI the luminous output, in lumens, of several possible light sources, each with a total power output of one milliwatt. The radiation from <u>each</u> of these light sources, if focused at the retina could deliver a surface energy density of 1 joule/cm^2 in one millisecond. However, the characterization of the sources in terms of luminous quantities does not

TABLE VI

Luminous Output of Various One Milliwatt Output Light Sources

Incandescent Lamps		.16 Lumens
GaAs, Semiconductor Laser	$\lambda = 8900\text{Å}$	0 Lumens
HeNe Laser	$\lambda = 6328\text{Å}$	.17 Lumens
Ionized Argon Laser	$\lambda = 5145\text{Å}$	.4 Lumens
	$\lambda = 3002\text{Å}$	0 Lumens

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indicate the similarity of danger presented by each.

In biophysics where prime consideration is given to physical phenomena, such as damage thresholds, photochemical interactions and thermal interactions, the energy and power of radiation must be unambiguous or at least easily understood.

In studies primarily involved with physiological problems either unit system can be used. Due to the vast amount of work that has been done in the past, (for example reference 5), on the physiology of vision, where illumination conditions are described photometrically, it seems convenient to maintain the status quo. It would be advantageous if such studies could indicate the P of the light source involved, since the interrelation between physiological and physical phenomena is of interest (5,6,7,8). This does imply additional work, but the methods of determining P are well known (9). In addition, particularly in visual effects and hazards, it is interesting to compare reversible and irreversible hazards; the crossover point from flash blindness to retinal damage is of interest in laser research. However, the work done on flash blindness uses the photometric system almost exclusively (12).

Retinal damage, thresholds are characterized generally in terms of radiometric units. Kinsey et. al. (15) uses photometric measurements of the visual output of an electric welding arc to indicate the amount of ultraviolet radiation present. This method is, at best, dubious and any attempt to relate these measurements to a physical threshold value for flash keratoconjunctivitis is equally suspect. The only apparent worthwhile information that can be gleaned from this work of Kinsey is that the threshold for onset of a photophthalmic reaction in humans is about one half that

of rabbits and one fourth that of dogs.

Indeed, improper use of radiometric units occurs in the literature also (10,14). The use of the unit erg-sec/cm^2 by Duke-Elder (14) and repeated in project Agile (10) when referring to previous work can cause confusion. In one instance the unit is used as a unit of power and in a second case it is used as an energy unit.

Not only can the photometric units be confusing but the misuse of the simpler radiometric ones must be avoided.

To summarize, the radiometric unit system should be used in describing biophysical phenomena under exposure to optical radiation; the reasons for this are as follows:

1. The units are fundamental and give a complete, unambiguous description of the radiation.

2. Correlation between incident radiation and physical observables such as temperature changes, are done easily when the radiation characteristics are given in radiometric units.

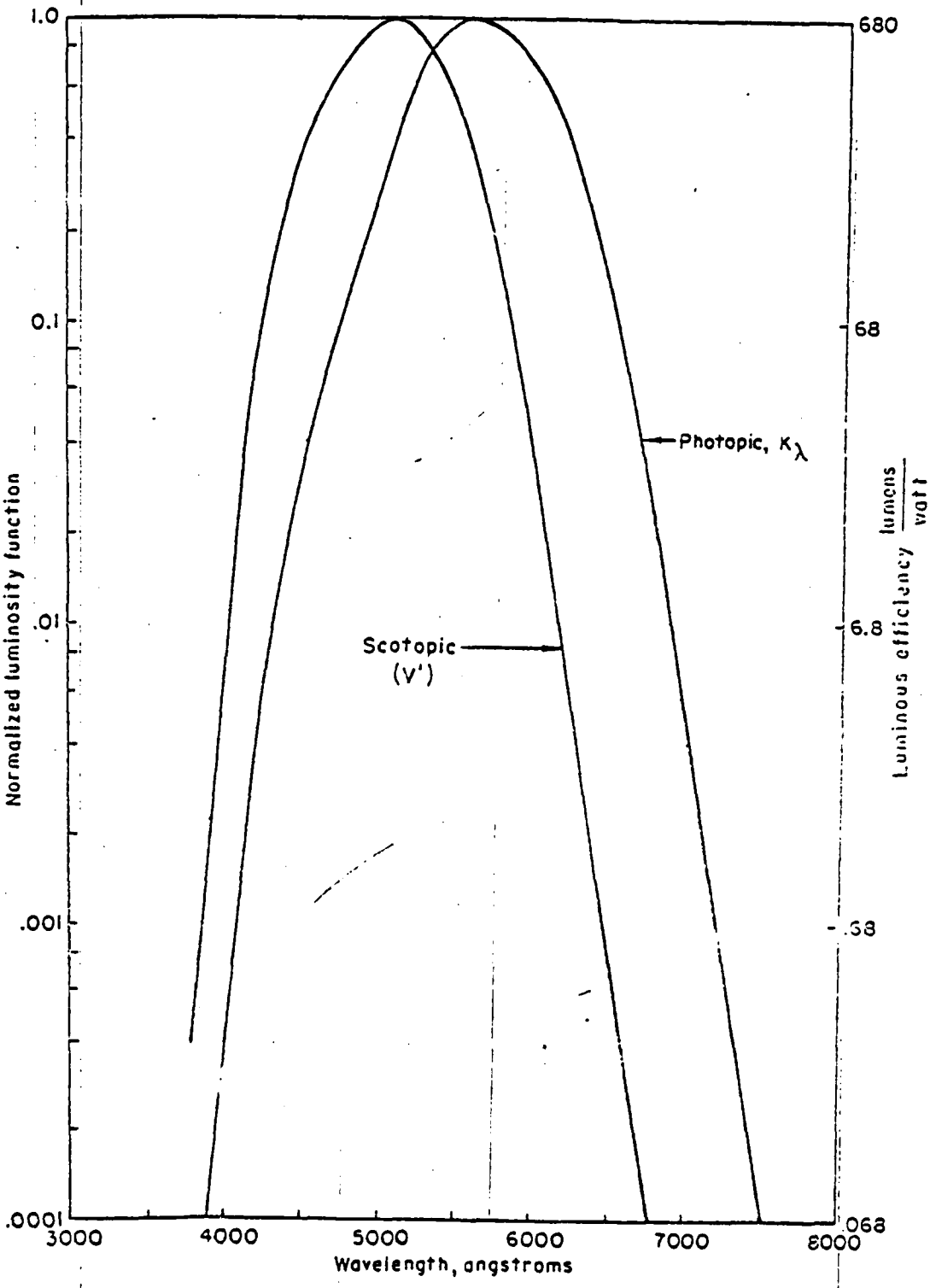
3. Radiometric measurements are not limited in wavelength as are photometric units. Radiation beyond the visible region can only be described in radiometric units.

4. A comparison of physical effects initiated by various sources of radiation (such as broad band Xenon flashtube radiation and narrow band laser radiation) can be done more readily only in radiometric units. Other factors such as the relative attenuation by ocular media, absorption, scattering and attenuation must be considered.

5. Comparisons or relations between physiological and physical effects cannot be done by photometric descriptions. Radiometry must be used since physical effects can be described quantitatively only in radiometric units.

6. Ophthalmological hazards of lasers can be described by both unit systems but the radiometric is to be preferred since a few numbers may be used to classify hazard levels for a wide variety of laser wavelengths. If photometric units are used, individual hazard levels must be specified for each wavelength.

The problem of whether the time for reversible visual impairment is a function of the wavelength has not been considered. If it is independent of wavelength, then radiometric units will adequately describe both reversible and irreversible impairment. If reversible impairment is a function of wavelength, then a curve relating reversible impairment to wavelength must be considered in conjunction with the radiometric units.



Photopic and scotopic visible sensitivity curves

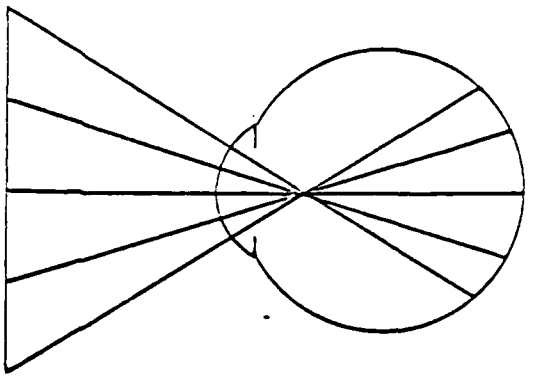


Figure II

REFERENCES

1. IES Lighting Handbook, 3rd Edition; Illuminating Engineering Society New York, N.Y., (1959)
2. Photometry, 2nd Edition; J.W.T. Walsh, Constable & Co., L.I.D., London
3. Levi, L., "A Short Course in Photometry", Electronics Products; 16 (1965)
4. J. Optical Society of Amer., 52 (4); 490, 1962
5. Enoch, J.M., "Optical Properties of the Retinal Receptors", J. Optical Soc. Amer., 53 (71), 1963
6. Boynton, R.M., "Quantun and Energy-Bared Visual Sensitivity on a Single Plot", J. Optical Soc. of Amer., 53 (641), 1963
7. Wolf, E., Zigler, M.J., "Dark Adaption Level and Size of Testfield", J. Optical Soc. of Amer., 40, (211), 1950
8. Southall, J.P.C., Introduction to Physiological Optics, Oxford University Press, London, 1937
9. Christensen, R.L., Ames, I., "Absolute Calibration of a Light Detector", JOSA, 51, (224), 1961
10. Christner, C.R., et. al., "State-of-the-Art Study on Visual Impairment by High-Intensity Flash of Visible, Infrared, or Ultraviolet Light", Report No. BAT-171-9, Battelle Memorial Institute, Jan. 1965
11. Light Coagulation by Gerd Meyer-Schwickerath, C.V. Mosby Co., St. Louis, Mo., (1960)
12. Sperling, H.G., "Flash Blindness as a Function of Wavelength Specificity", Fed. Proc. Vol. 24, No. 1, Part III, p. S-73, 1965

13. Moon, P., Spencer, D.E., "On the Stiles-Crawford Effect", JOSA, 34,(319), 1944

14. Duke-Elder, S., Textbook of Ophthalmology, Vol. VI, C.V. Mosby Co., St. Louis, Mo. (1954)

15. Kinsey, V.E., et.al., "Measuring Eye Flash from Arc Welding", J.A.M.A., 123(7), (403), 1943

Laser Eye Protection

Recent investigations have indicated that direct viewing of both pulsed and C-W lasers can result in either reversible or irreversible disturbances in vision. Protection of personnel from these hazards has been the subject of a number of papers (1,2,3,4,5). These studies have been oriented toward protection in the research laboratory and industry, as well as in the field.

The extensive investigations by Ham et al. (6,7,8,9) on threshold studies for the production of lesions in rabbit fundi, where thresholds were determined by ophthalmoloscopic examination after irradiation by carbon arc, xenon lamp, or pulsed ruby laser, has been widely used as a basis for estimation of the safe laser dose to the human eye. These studies indicate that a mild, irreversible retinal lesion was ophthalmoscopically visible five minutes after irradiation by a ruby laser pulse of 200 μsec duration at a calculated energy density of 0.7 joules/cm^2 at the retina. This radiation dose is approximately the same as that required from conventional white light sources (xenon lamps and carbon arcs) for exposure times of 200 μsec. In addition, at this exposure time, the threshold energy density showed no dependence on retinal image diameter for spots ranging from 1.05 mm to 0.54 mm diameter (8). A simple thermal model of the fundus indicated that, for exposures of 200 μsec duration, very little energy is lost due to radial heat conduction away from the irradiated site during the pulse(8).

Straub (1,2) has considered several methods of providing protection for the human eye from laser radiation, both in the laboratory and in the field. He has suggested that warning can be given before a laser is

fired in the laboratory, thus giving personnel sufficient time to close their eyes. It is probable, however, that as the power and energy of lasers increase the human eyelid will not provide sufficient opacity to protect the eye from direct laser radiation or even scattered laser radiation. Precautionary measures such as covering the eyes with the forearm, may provide additional protection. Further study in this area is warranted.

In the field, where friendly and enemy lasers may be continually operating, warning cannot be given to personnel. This situation necessitates devising a method for continuous eye protection. Methods considered by Straub include the automatic, high speed shutter triggered by the arriving laser pulse, and colored absorption filter or dielectric reflection filters. He disqualifies Kerr cell shutters because of their small angle of acceptance and low optical density in the fully closed state. Phototropic filters acting as optical shutters from stimulus in the far red with an appropriately short response time (10^{-8} sec) and sufficient opacity were not available at that time. Such devices do not appear to be available in the literature at present.

In his well documented studies, Straub considers dielectric reflectors or colored absorption filters as better possibilities for eye protection in the field. Dielectric reflectors are rejected, however, due to the angular dependence of effective reflectance. Straub assumes that the hazardous radiation associated with ruby lasers lies in a narrow band in the far red. Thus it is possible that colored absorption filters can be constructed so as to pass near red and visible radiation (to allow visual observation of monitoring equipment, etc.) and yet protect the wearer against direct viewing of laser light. Such filters may however not be completely satisfactory, at present, for a number of reasons:

1. The source of ocular hazard to personnel does not necessarily lie at the initial laser wavelength. Intense light from backscattered plumes, as well as coherently scattered harmonic frequencies in the visible range, is a distinct hazard to the wearer of such glasses.

2. Under field conditions, laser frequencies being employed by the enemy may not be known. Protective glasses with fixed abosrption bands are of little value against narrow band incident laser light of unknown frequency.

Using Ham's result that an energy density of 0.2 cal/cm^2 delivered to a rabbit retina in 175 μsec. produces a mild, irreversible lesion, observable three to five minutes after exposure, Straub concludes that 1.6×10^{-6} joules at the iris, focused to a diffraction limited spot at the retina (2×10^{-6} cm^2 when the dark adapted iris is the limiting aperture) is the least energy required to produce irreversible retinal damage. Whether diffraction limited spot size is justifiable merits further consideration. A statistical approach to the problem of irreversible threshold damage may be necessary. One problem associated with a consideration of a diffraction limited spot size is that the extrapolation of the data of Ham et al. to one diffraction limited may not be justifiable. Radial heat conduction away from the irradiated site may occur and must be taken into account for a diffraction limited spot size. It is possible that the assumption of a diffraction limited spot may not represent the situation where the total energy required to produce irreversible damage to the retina is a minimum. An irreversible lesion of a diffraction limited spot size may not be the most disabling.

According to Ham et al. (10), heat dissipation by conduction at the image borders will have an appreciable effect on the temperature at the center of a 20 micron diameter spot at the retina, in approximately 180 μsec. Straub's extrapolation to a 16μ diffraction limited spot diameter may therefore, be acceptable for shorter laser pulse durations. However, experimental verification of this model for a diffraction limit spot is desirable.

If it is assumed that the diameter of a perfectly collimated laser beam is larger than the pupillary aperture of the eyes, then the diffraction limit of the retinal spot will be determined by the pupil diameter D_p. Since the diffraction limited spot diameter is then given by

$$H = \frac{2F}{D_p}$$

where H = Diameter of retinal image spot

F = Focal length of the eye

= Wavelength of incident radiation

Dp = Pupil diameter

It is apparent that, for a given focal length (given eye accomodation), the diameter of the image spot at the retina will be a minimum when D_p is a maximum (i.e. when the eye is completely dark-adapted). It should be noted that a perfectly collimated incident beam will be focused at the retina only when the eye is accomodated for viewing a distant object. For any other accomodations, the retinal image from a collimated beam will be larger than the diffraction limit.

The minimum energy required to produce irreversible retinal damage occurs for a diffraction limited focal spot and maximum pupil diameter. Straub has then chosen the case necessary to produce an irreversible lesion for 175 μsec pulsed lasers operating in the far red, where the ocular transmission to the retina is almost 100% (11). If one considers longer pulse lengths, and/or a spot size larger than the diffraction limit, the least energy at the pupil required to produce threshold irreversible damage to the retina occurs for the smaller spot sizes (8).

Swope and Koester (4) have published calculations of the attenuation required to protect the human eye against pulsed laser radiation. These calculations are based on published data (5,6) on threshold dosage for an observable retinal lesion in rabbits, and data concerning human (in vitro) ocular transmission characteristics. The threshold energy theoretically required to produce a retinal lesion in the human eye is determined for both ruby and Nd-glass lasers with a given beam divergence. One of the assumptions on which the studies by Swope and Koester (as well as that of many others) are based, is that human retinal damage will occur at the same power densities as that required to damage a rabbit retina. They conclude that a pulsed ruby laser with a beam divergence of $1/2^{o}$ will produce irreversible retinal damage to the rabbit (and hence human) when the entire beam enters the eye with an energy of 10^{-4} joules. This result is a factor of 100 greater than that obtained by Straub for diffaction limited retinal images produced by a collimated beam.

Suitable eye attenuators are discussed (Swope and Koester). They conclude that a combination of BG-18 and BG-38 glass, each 2 mm thick, provides the best eye protection with minimal hazard due to crazing of the glass. A nomograph is then devised whereby a "Safe Laser Output"

is determined for personnel wearing BG-18 and BG-38 combination safety glasses. The nomograph is based on the assumptions that: 1). a suitable safety factor can be established so that the threshold energy for retinal damage to the rabbit can be correlated to the human eye; 2) all light from the laser enters the eye; 3) the area of the illuminated retinal spot depends only on θ^2, where θ is the beam divergence as it enters the eye, when the eye is focused at infinity.

Adequate data is required as to the correlation between the human and rabbit eye. The importance of physiologic and anatomic differences between the rabbit and human eye must be considered. It is, therefore, difficult to assign a definitive value to threshold lesion data taken from studies on various rabbits.

The assumption that the illuminated retinal spot diameter depends only on the beam divergence angle when all light enters the eye, is true only under certain conditions. In addition, if the eye is a distance L from a laser aperture of diameter d and if the focal length of the eye is F, then:

(a) When $L - (F + \frac{d}{\theta}) > 0$ the minimum spot lies in the image plane of the eye (retina)

(b) When $L - (F + \frac{d}{\theta}) = 0$ the beam diverges within the eye

(c) When $L - (F + \frac{d}{\theta}) < 0$ the minimum spot lies in the focal plane

It is important to realize that retinal spot diameter depends on eye focus. The assumption that the eye is focused at infinity only gives a minimum spot diameter at the retina in case (c) (and the diffraction

limited case). If the eye is focused at infinity in case (a), the minimum spot will lie behind the retina. If, however, the eye is focused at the laser aperture in case (a), the minimum spot will lie on the retina. Therefore, in case (a), a "safe laser output energy" that is calculated for the eye focused at infinity will no longer be safe if the eye is focused upon the laser aperture.

In summary, parameters, such as laser aperture, eye-to-laser distance, eye focal length, as well as beam divergence, must be considered in a theoretical model for damage to the human retina. Damage to other than the retina must also be considered, particularly as other wavelengths become available.

Although many of the factors associated with protective glasses, particularly BG-18 and BG-38, have been extensively studied by Straub (1,2) and by Swope and Koester (4), further investigation is necessary, particularly as power and energy density levels, and other wavelengths become available.

REFERENCES

1. Straub, H.W., "Protection of the Human Eye from Laser Radiation", Army Materials Command, Report TR-1153 (Harry Diamond Laboratories) 1963.
2. Straub, H.W., "Use of Protective Goggles in Areas of Laser Radiation" Fed. Proc. 24 (1) Part 3, Suppl. 14, S-78, 1965.
3. Fine, S., et al. "Mechanisms and Control of Laser Hazards and Management of Accidents", Proc. 2nd Conf. on Laser Technology, Illinois Institute of Technology, Chicago, Illinois, April 1965.
4. Swope, C.H., and Koester, C.J., "Eye Protection Against Lasers" Appl. Optics 4, 532, 1965.
5. Solon, L.R., Aronson, R., Gould, G., Science 137, 1506, 1961.
6. Ham, W.T., Jr., et al., Amer. J. Ophthal. 43, 711, 1957.
7. Ham, W.T., Jr., et al., Amer. J. Ophthal. 46, 700, 1958.
8. Ham, W.T., Jr., et al., Acta Ophthal., Suppl 76, 60, 1963.
9. Ham, W.T., Jr., et al., Acta Ophthal., (Submitted for publication).
10. Ham, W.T., Jr., et al., "Ocular Effects of Laser Radiation, Part I" Defense Atomic Support Agency Report DASA-1574.
11. Geeraets, W.J., and Ridgeway, D., "Retinal Damage from High Intensity Light", Acta Ophthal., Suppl. 76, 109, 1963.

Laser Dosimetry - Biological Systems

Few studies have been reported on laser dosimetry. Fine et al. (1) have discussed the difficulties involved with laser dosimetry from both a physical and biological standpoint. Schlickman and Kingston, and Schlickman and Diffley have reported the development of a pulsed laser dosimeter for ocular hazards measurements (2,3).

The Schlickman - Kingston dosimeter utilizes a modified gamma-radiation dosimeter as a high-impedance voltmeter. The instrument is calibrated with a steady state light source and an optical spike filter so as to read full scale when an energy density believed to be capable of causing retinal damage to the human eye enters the instrument at a specified wavelength.

The instrument is used in the following manner. A laser target is selected and the diameter of the area to be irradiated on the target is determined by first irradiating a piece of carbon paper or exposed Polaroid film. The distance from the target to the anticipated position of the eye of an observer is measured. The aperture of the dosimeter is adjusted from a chart (which appears in the article) to yield a full scale deflection for a hazardous radiation dose. Schlickman and Kingston point out that the target area is then illuminated by an incandescent source and the scattered light focused to a sharp image (by means of a viewing system attached to the dosimeter which they describe) on a diffusing plate that lies at the entrance to a phototube within the dosimeter. The laser is then fired at the target and a reading taken on the dosimeter. A reading of 1/10 scale is assumed to indicate unsafe viewing of the laser impact by the unprotected eye.

The authors explain that the instruments dynamic range encompasses all known, non-Q-switched lasers operating in the 6,000 to 10,000 Å range. Provisions have not been made for work with Q-switched lasers. This is due to design difficulties and the lack of quantitative biological data regarding such lasers. The dosimeter will accurately respond to laser pulses of at least 25 μsec duration composed of triangular spikes at least 400 μsec wide at the base with approximately a 1 μsec delay between the end of one spike and the beginning of the next.

To set the level of "safe" radiation exposure for the human eye, Schlickman and Kingston have incorporated the threshold data of Ham and Geeraets (4). Schlickman and Kingston have used a safety factor of 10, in correlating this data with the hazardous radiation dose to the human eye. However, due to the lack of quantitative, in vivo data for correlation of the physical and physiological characteristics of various strains of rabbit and human eyes this safety factor requires further consideration.

The general effectiveness of single pulse laser dosimetry is doubtful under normal laboratory conditions. Basic to the work of Schlickman and Kingston is the assumption that, if a dosimeter reading in the field of scattered laser light indicates a safe level for a given laser burst, then a second laser burst at a similar target will also produce scattered light at a safe level. The dosimeter cannot measure the level of either direct or scattered laser radiation reaching the eyes of laboratory personnel during a specific laser pulse. Safe radiation levels can only be inferred from dosimeter measurements that are made prior to the exposure of personnel to

scattered (or direct) laser radiation. There are many instances where the target surface to be irradiated is not the same from shot to shot (eg. biological targets). Changes in target surface will change the patterns of scattered light intensity and thus reduce confidence in predicting scattered light intensity at a specific location in the laboratory for subsequent laser pulses. With many pulsed lasers, output power density is not easily reproduced from pulse to pulse and should be considered as a factor in evaluating a single pulse laser dosimeter.

The Schlickman-Kingston dosimeter is equipped with an adjustable iris that reduces the total radiative energy entering the dosimeter for scattering conditions that will produce a spot on the retina that is larger than the diffraction limit of the eye. Further data and a theoretical model of the eye would be desirable to explain the curves shown in the article. A more complete theoretical treatment of the focusing of specularly scattered light by the human eye would be of interest.

In view of radial heat conduction within the pigment epithelium, the retinal spot diameter as well as area is an important parameter for certain laser pulse durations (5). Using xenon sources, Ham et al. (6) have shown that for pulses from approximately 100 μsec to 1 msec duration, radial heat conduction is relatively unimportant even for diffraction limited spots on the retina. Hence, for pulsed lasers operating in the 175 μsec - 1 msec range, one can consider the threshold energy required to permanently damage the retina as being a function of only the retinal spot area. This being the case, it becomes important to know the area of the human retina that is actually irradiated by scattered light from a target surface.

A factor of importance with respect to the Schlickman-Kingston dosimeter is that it is only calibrated over a narrow band near a desired laser wavelength. There is no reason to assume that all the hazardous scattered radiation will be confined to that frequency band. Brilliant, broad band plasmas have been observed on laser radiation of both physical and biological targets (7,8). These plasmas are generally small and may be focused to a small image on the retina of an observer. The S-1 response of the Schlickman-Kingston dosimeter is relatively insensitive in the blue-green spectral regions, the response at 5000 Å being 20% of maximum. The biological effects of viewing laser generated plasmas has not been investigated and should be considered as a potential hazard. Scattered light may also contain frequency doubled components. An S-1 dosimeter response will not yield reliable data for frequency doubled ruby or neodymium laser radiation.

The problem of monitoring laser radiation levels to determine safe conditions for personnel involves measuring several parameters. Power, power density, energy, wavelength, and anatomical site involved in laser interaction are some of the parameters that must be measured before a specific biological reaction to laser impact can be predicted. There is no single parameter that can be assigned to laser output that will also yield sufficient biological information to ascertain possible hazards. "Radiation dose" is a phrase that must be broad enough to include all parameters necessary to completely describe a specific biological reaction to a laser radiation field.

From the standpoint of instrumentation, it will be difficult to develop a simple monitoring dosimeter that can measure a sufficient number of physical and biological parameters to completely characterize the laser interaction.

Dosimetry for radiation from discretely pulsed lasers is difficult due to variations in scattering, power output, beam heterogeneity, location of personnel and other biological and physical parameters from shot to shot. Extrapolation of data obtained from one laser pulse to the next is difficult. Fine et al. (1) have pointed out that if adequate instrumentation can be developed two types of dosimetry are desirable. First, instantaneous detection of unsafe conditions may be used for protection. Second, cummulative exposure measurements may be important for medico-legal records.

REFERENCES

1. Fine, S., et al. "Mechanisms and Control of Laser Hazards and Management of Accidents", Proc. 2nd Conf. on Laser Technology, Illinois Institute of Technology, Chicago, Illinois, April 1965.
2. Schlickman, J.J., and Kingston, R.H., "The Dark Side of the Laser", Electronics 38:8, 93, April 1965.
3. Schlickman, J.J., and Diffley, R.M., "Laser Dosimetry: The Design Engineer's Viewpoint", 1st Annual Biomedical Laser Conference, Boston, June 1965.
4. Geeraets, W.J., et al., "Laser Versus Light Coagulator: A Funduscopic and Histologic Study of Chorioretinal Injury as a Function of Exposure Time", Fed. Proc 24, Suppl. 14:1 Part 3, S-48, 1965.
5. Ham, W.T., Jr., et al., Acta Ophthal. (Submitted for publication).
6. Ham, W.T., Jr., et al., Acta Ophthal., Suppl. 76, 60, 1963.
7. Fine, S., et al., "Measurements and Hazards on Interaction of Laser Radiation and Biological Systems", NEREM Record, 1964.
8. Fine, S., et al., "Interaction of Laser Radiation with Biologic Systems, I. Studies on Interaction with Tissues", Fed. Proc. Suppl. 14, 24, Part 3, S-35, 1965.

Polarization of Lasers - Effects on Energy Measurements

In most energy measurements of laser radiation, the output intensity is often sampled by a low reflectance beam splitter. This procedure is usually followed when it is desired to monitor the laser energy during each pulse or when the full laser energy would destroy the monitor. Generally the beam splitter has been placed at a 45° angle to the optical axis, reflecting a small fraction of the light to an energy measuring device while most of the beam is transmitted to the target. The angle of 45° has been chosen, since it permits placement of the energy measurement device at 90° to the beam axis. This procedure is common in bio-physical laser research, where various beam splitters are used (1,2).

There is a very important factor that is not usually considered in estimating the reflectance of the beam splitter. Depending on the angle at which the beam splitter is set, the polarization properties of the laser beam can be responsible for errors in excess of 100% of the calculated reflectance. In this section the source of these errors and a method for minimizing them will be shown. For the sake of brevity only single surface reflections will be considered.

Most optical phenomena can be described in terms of electromagnetic theory. In the wave model of light, a light beam is considered as a transverse wave with perpendicular electric ($\vec{E}$) and magnetic ($\vec{H}$) field vectors. The simplest type of electromagnetic wave is one that can be expressed in terms of a sine or cosine function. For example, using the cartesian coordinate system, an electromagnetic wave traveling in the

positive x direction could have its electric field vector in the y direction. in which case:

$$\vec{E} = \vec{E}_y \cos(\omega t - kx)$$

and

$$\vec{H} = \vec{H}_z \cos(\omega t - kx)$$

where

$$\vec{E}_x = \vec{E}_z = 0$$

$$\vec{H}_y = \vec{H}_x = 0$$

and

$$\omega = 2\pi f$$

$$k = \frac{2\pi}{\lambda}$$

where

f = frequency, and λ = wavelength

A wave which maintains this orientation is said to be plane polarized or linearly polarized. Indeed, any light wave in which the electric (and thus the magnetic) field vector maintains a constant orientation is said to be plane polarized. Most optical radiation is unpolarized, that is the individual wave trains have their electric field vectors oriented in a random fashion, giving the overall effect of having every possible linear polarizations at once.

Linear polarization can be selected from unpolarized light by passing the unpolarized beam through a polarizer. Unpolarized light can be theoretically considered as consisting of two mutually perpendicular polarizations. This is because, once an orientation is chosen, all the other vectors can be decomposed in the ordinary fashion into two mutually perpendicular components. Unpolarized light may be represented

by two orthogonal vectors depicting the electric vector orientations as shown in Figure I.

A polarizer selects one polarization. If two polarizers are set to accept perpendicular polarizations, then their combined action on a beam is to pass nothing at all, as is shown in Figure II.

Many practical polarizing devices are made from crystals which naturally have the physical properties of birefringence or double refraction. Many other materials exhibit birefringence when placed under mechanical stress for example polaroid plastic, or under electrical stress, for example the Kerr effect, or under magnetic stress, for example the Voigt effect.

The ruby used in ruby lasers is naturally birefringent and the output of ruby lasers has been shown to have a varied polarization (3,8) Most gas lasers operate with a linearily polarized output due to the design of the discharge tube (the Brewster angle windows will be commented on further in this section). Polarized outputs have been observed in gas lasers without Brewster angle windows however. This fact may be due to a mechanical stress birefringence in the glass itself or induced magnetic birefringence (4,5). The output of Neodymium in glass may have various polarization properties. However, studies do not appear to have been carried out in any detail in this area. A review of the contradictory observations on the polarization of light emitted from GaAs laser diodes has been carried out by Nannichi (9). His study also showed that the polarizations of the GaAs laser radiation shifted its orientation as a function of current. No cause of the polarization was found (9).

The major point to emphasize concerning laser polarization is the fact that those lasers which have been investigated are polarized to various extents. The polarization in ruby not only changes from shot to shot, but even varies during a single shot (3). There is almost no information on the Neodymium laser, but there is no a priori reason to expect uniformly oriented polarization while the possiblities of random polarization may exist (4,5). The following analysis thus directly applies to ruby lasers and may well be significant for the case of Neodymium in glass.

The basic optical law of refraction, Snell's law states that if a light beam is incident at the interface between two media at an angle θ relative to the normal to the surface, then the beam will be refracted in the second medium, at an angle of θ' from the axis, and that the ratio of the sines of the two angles is the inverse ratio of the indices of refraction of the two media.

$$\frac{\sin \theta}{\sin \theta'} = \frac{n'}{n} \qquad \text{SNELL'S LAW}$$

However, not all the light is transmitted into the second region, some is reflected at the interface at an angle equal to the angle of incidence, Figure III.

The Fresnel laws of reflection give the reflection and transmission coefficients for the various beams depending on their polarization. Any beam striking the surface can be either linearly polarized, with its electric vector perpendicular to the plane of incidence or parallel to

the plane of incidence (see Figure III), or unpolarized and representable as a linear combination of the two linear polarizations (elliptical and circular polarization must also be considered).

The reflectances are a function of the angle of incidence and the angle of refraction and may be written as (7):

$$r_p = \frac{\tan^2(\theta - \theta')}{\tan^2(\theta + \theta')}$$

$$r_s = \frac{\sin^2(\theta - \theta')}{\sin^2(\theta + \theta')}$$

where the subscripts:

p = parallel polarization

s = perpendicular polarization

and where the angle of refraction, θ', must be calculated from Snell's law.

Ordinarily, the reflection coefficient of a surface is calculated for unpolarized light in which case the average of the two polarized reflectances is used (5). The average reflection coefficient is written as

$$r_{av} = \frac{1}{2}(r_p + r_s)$$

Figure IV is a graph showing the three reflection coefficients for air to glass, (high dispersion crown at 6560 Å), n = 1, n' = 1.517 as a function of angle of incidence. It should be noted that at 56.58° the reflectance of the parallel component goes to zero. This is the Brewster angle. At this angle the parallel polarization is transmitted with no

reflection loss. In gas lasers the use of windows set at the Brewster angle causes the parallel polarization to be favored in the amplification process.

The reflection coefficient for either polarization depends on the angle of incidence and the ratio of the indices of refraction between the two media. If an air-glass (or quartz) interface is assumed, the variation of reflectance for $\theta = 0^{\circ}$, as a function of refractive index, can be specified in terms of the refractive index of the glass (air is assumed to have an index of one). This dependence is shown in Figure V. This curve is calculated from the refractive indices of crown and flint glass and quartz for various wavelengths (5). Thus the refractive index of a given beam splitter, hence the reflection coefficient, is a function of the material used and the wavelength of the laser beam.

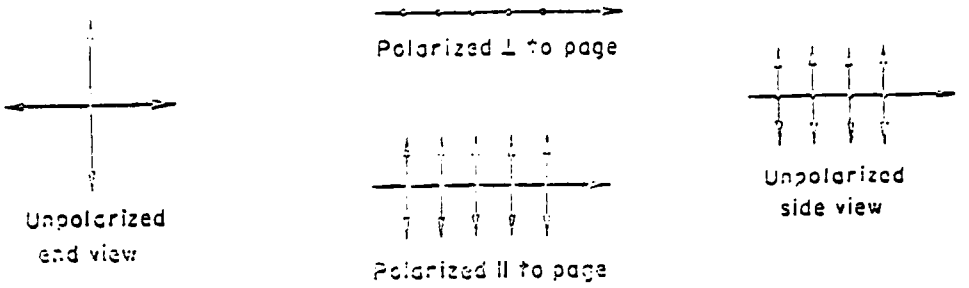
Figure VI shows the error that can exist due to beam polarization if the average reflection coefficient is the value specified. The error is plotted as a function of angle of incidence. At 45°, the most commonly used beamsplitter angle, the error due to perpendicular polarization is about +5% and that due to the parallel polarization has a value of -70%. It is reasonable to conclude that if the average reflectance coefficient for a beam splitter is used, then the energy of a laser beam with varying polarization cannot be measured accurately by the beamsplitter technique, as ordinarily used.

As an example, consider a single surface beam splitter ($n' = 1.517$) set at an angle of 45° to the beam axis ($\theta = 45^\circ$). The average reflection coefficient is 5.3%. The reflection coefficient for parallel polarization is 1% while that for perpendicular polarization is 9.3%. A one thousand joule, parallel polarized laser beam would be diagnosed as having an energy of about 200 joules if the average reflection coefficient were used. Similarly a reflected energy of 5 joules could be interpreted as 95 joules, 55 joules or 500 joules according to whether the average, perpendicular or parallel reflectance coefficient were used.

It is, thus, most important that the polarization properties of the laser beam be taken into account when reflection dependent beam sampling methods are used. A beam splitter set at 45° can produce large errors in energy measurement. Since the polarization properties of lasers are not widely explored, they must be considered as probable and of random orientation in the design of beam splitters.

A simple manner to overcome the ambiguity presented by various beam polarization properties is to use a low angle of incidence beam splitter. As can be seen from Figure VI by setting the beam splitter at an angle of 10°, the error introduced by beam polarization is less than 5%. This angular setting is recommended and should be followed unless some other technique of eliminating this source of error is found.

Due to the time factor involved these results must be viewed as highly preliminary. Further consideration must be given to the problem. The increased complexity due to reflections from both the first and second surfaces must be considered in detail. Experimental verification of the conclusions determined analytically must be carried out.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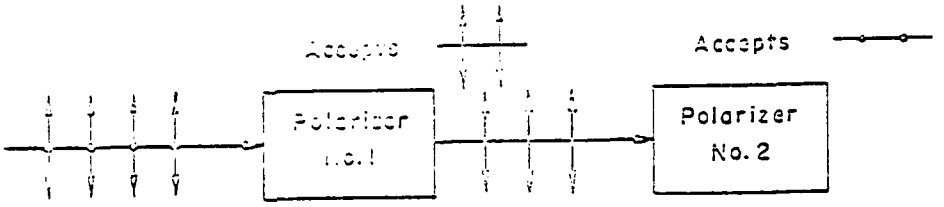
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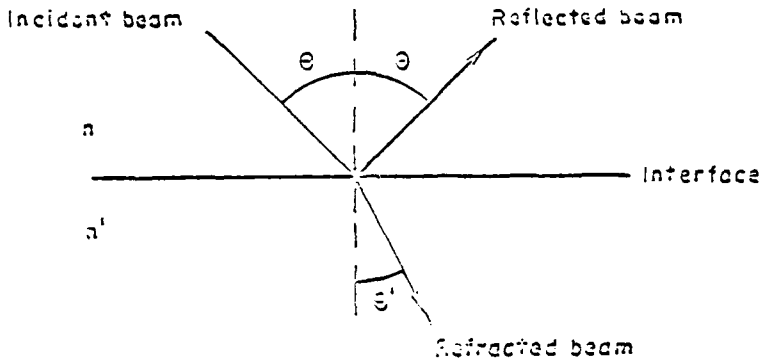
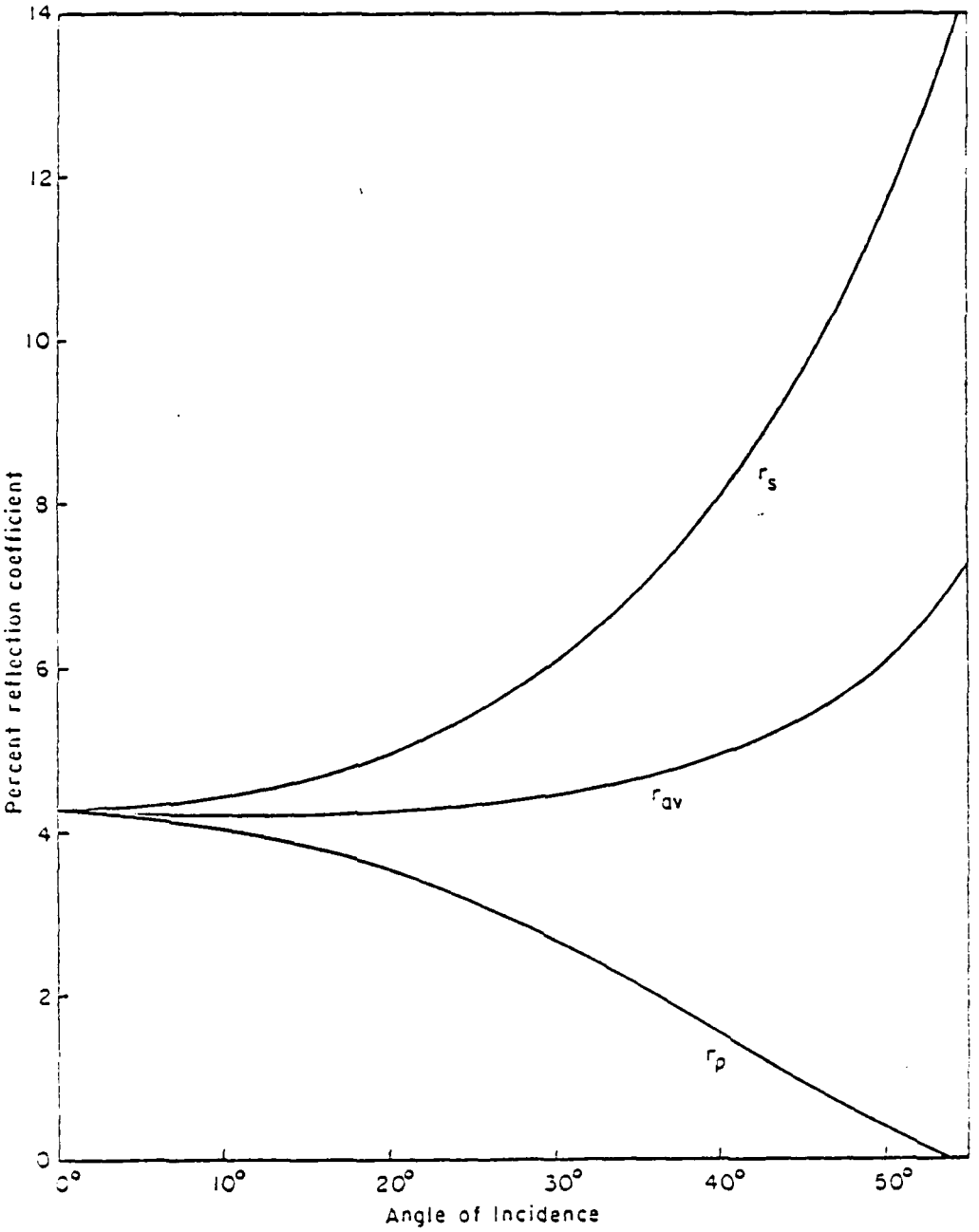
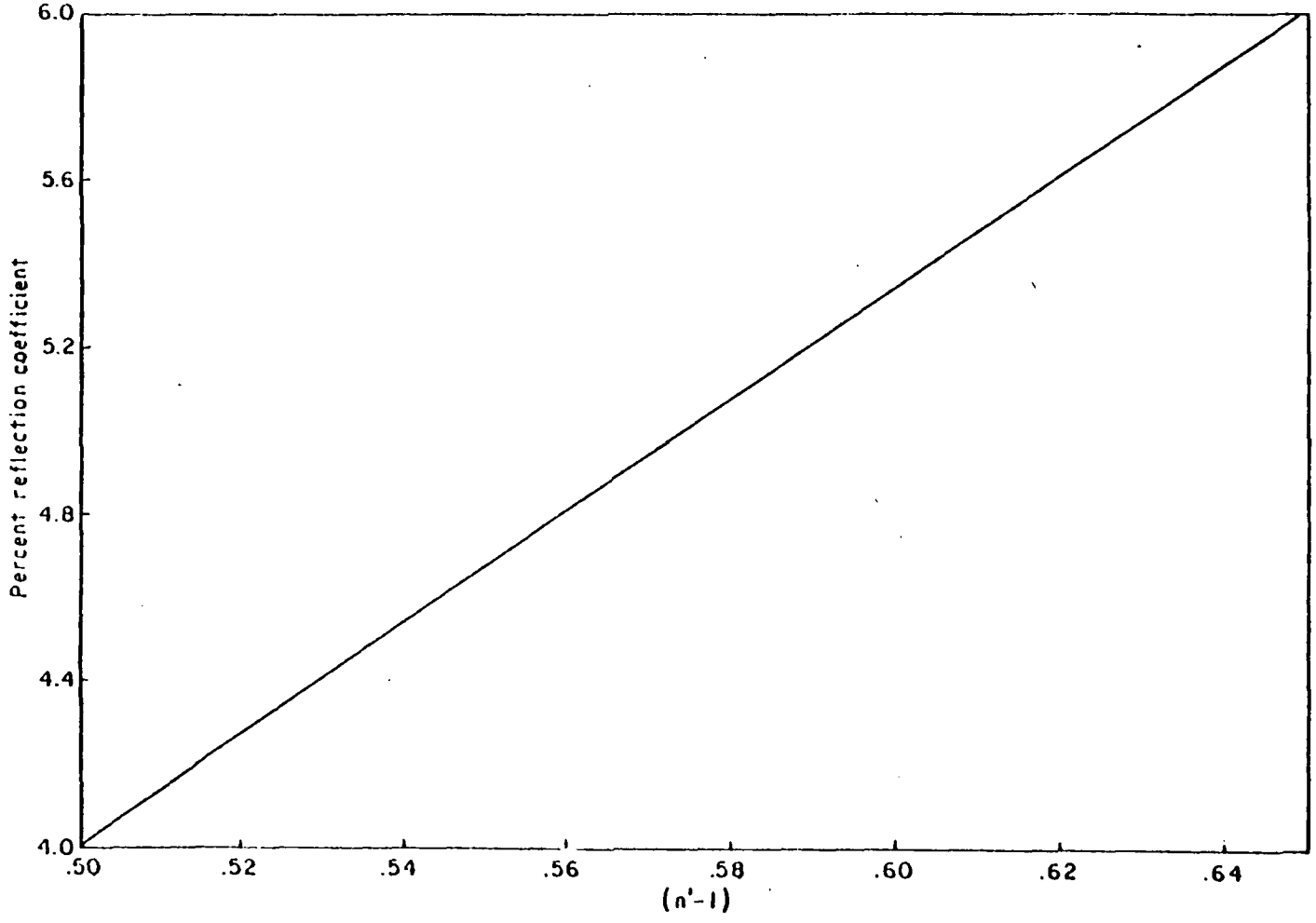


The plane of incidence is the surface of the page

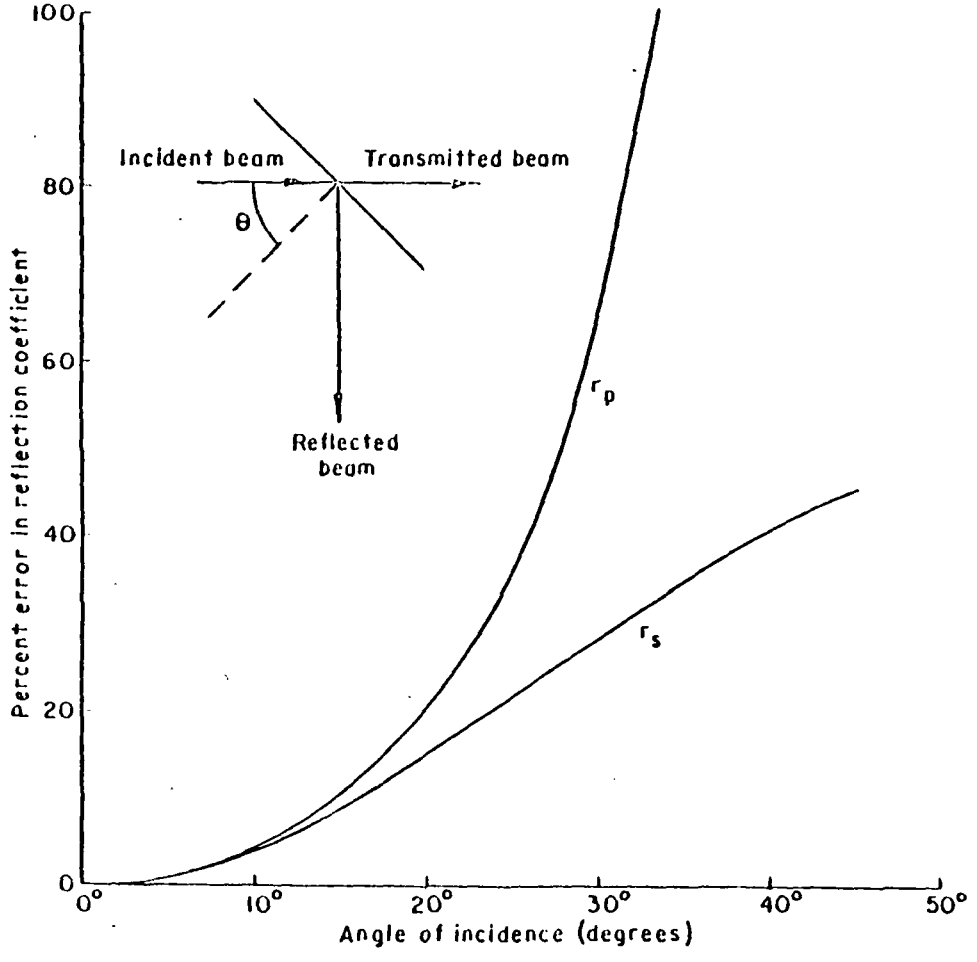
Geometry of reflection and refraction showing plane of incidence and the various angles.



Percent reflection of light from a single surface, n = 1.517 as a function of angle of incidence where r_s is for perpendicular polarization, r_p is for parallel polarization, r_{av} is for unpolarized.



Percent reflection at normal incidence versus index of refraction.



Error in reflection coefficient versus angle of incidence for an assumed average reflection coefficient if incident light is polarized, r_p if parallel, r_s if perpendicular (n' = 1.517).

References

1. Minton, J.P., et al, "The Effect of Neodymium Laser Radiation on Two Experimental Malignant Tumor Systems," Surg. Gynecol. & Obstetrics, 120:481, 1965.

2. Fox, J.L., Hayes, J R., and Stein, M.N., "The Effects of Laser Radiation on Intracranial Structures," 1st annual Biomedical Laser Conference, of the Laser Medical Research Found., Boston, Mass., June 1965.

3. Brunton, J.H , "Polarization of the Light Output from a Ruby Optical Maser," Appl. Optics, 3:1241, 1964.

4. Doyle, W.M, and White, M.B., "Frequency Splitting and Mode Competition in a Dual-Polarization He-Ne Gas Laser," Appl. Physics Lett., 5: 193, 1964.

5. Handbook of Chemistry and Physics, 45th Ed.,(The chemical Rubber Co., Cleveland, Ohio, 1964-65).

6. Javan, A., Ballik, E.A., and Bond, W.L., "Frequency Characteristics of a Continuous Wave He-Ne Optical Maser," JOSA 52:961, 1962.

7. Jenkins, F.A., and White, H.E., "Fundamentals of Optics. (New York: McGraw-Hill, 1957).

8. Tamai, T., and Achiwa, M., "Polarization of a Ruby Laser Beam," Japan. J. Appl. Phys., 3:60, 1964.

9. Nannichi, "On the Polarization of Light from the GaAs Diode Laser," Japan. J Appl. Phys., 3:360, 1964.

Discussion of Some Laser Output Detectors

Instruments available for measuring the output of lasers fall into four primary catagories:

1. Those which convert the output to heat and measure the temperature change of an absorber.
2. Those which sample the output beam directly, as in photodiodes.
3. Those which convert the output to mechanical energy.
4. Those which convert the output to chemical energy.

This section is concerned with the first two categories since these are the most usual methods employed in commercial calorimeters. Some general remarks, however, apply to all classes of laser radiation detectors.

Whenever radiation is incident on matter, there is a reflection which is dependent both on the parameters of the radiation, including wavelength and polarization, and on the characteristics of the reflecting medium, such as surface texture and the reflecting properties as a function of wavelength. In general, the polarization of a laser beam is not well defined (1). Consequently it is difficult fo predict the properties of the reflected radiation. Hence the transmitted radiation cannot be accurately characterized. Furthermore, there is no evidence that the parameters of a laser beam, such as polarization and beam homogeneity, are constant from pulse to pulse or during any given pulse.

In devices which transform the output to heat (thermal energy), the sensing device is usually an easily calibrated heat sensor. This sensor is in contact with a medium which is heated by the incident radiation it absorbs. The temperature rise is related to the absorbed energy by a

response curve which is accurate only for specific values of absorption, reflection, temperature distribution within the receiver, and in some cases ambient or reference temperature. These and some other parameters will be discussed below.

In most of the devices which transform the incident energy to heat there are two components; the energy absorber, and a temperature sensor (2,3,5,6). The energy absorber is generally a device employing the Mendenhall Wedge Effect. Briefly the Mendenhall Wedge Effect is the production of many reflections in a wedge or cone shaped enclosure of specified absorptivity (Fig. 1). The absorptivity of a medium is the fraction of incident radiation absorbed by that medium. The incident energy is strongly attenuated within a cavity employing this effect. If the end is not sharply pointed, fewer reflections take place, and consequently less energy is absorbed (Fig. 2).

Usually a small angle cone is employed as the cavity. Daly (2) has calculated greater than 95% absorption due to 15 reflections of the radiation in a 12 degree cone, with light incident on the cone aperture parallel to the axis, and with the cone having a specular reflectivity of the order of 80%. His data does not include the cases of either non-parallel light entering the cone, nor the cone being a diffuse, rather than polished, reflector.

Eisenman et al (3) have reported greater than 95% absorption in the infrared by using blackened cones. Their experiment was done by using the two extreme cases of parallel light entering the cone, and a point source of light situated on the axis of the cone. Their cone was assumed to be a perfect specular reflector, and they therefore do not consider the case of a diffuse reflector. Gouffé (4) and others have analyzed the effective

absorptive cavity of a conical receiver which has a perfectly diffusing reflecting wall and in which the incident energy enters as a collimated beam parallel to the cone axis. Although this may be an important theoretical case, it is usually not employed in practice, since the coatings generally employed to increase absorptivity, such as carbon, tend to cause specular rather than diffuse reflections. Therefore this paper will not consider that case. In a consideration of the case of a point source situated on the axis of the cone, the calculations carried out by Eisenman et al (3) are useful to investigators who focus the laser beam into the cone or who use diverging beams. However, the case of a beam focused so that the focal point lies beyond the point of the cone is not discussed.

In devices described by Ackerman (5) and by Dimeff and Neel (6) the problem of manufacture and maintenance of sharply pointed cones is circumvented by the use of stacks of razor blades, which also employ the Mendenhall Wedge Effect. However, a problem arises which is due to the dependance of the reflection coefficient on the polarization of the incident radiation.

All conductors have a complex index of refraction (21). The imaginary component of this number acts as an absorption term, but, since it is an imaginary number, it tends to change the phase of the light which it affects. Since it was seen in the section on polarization of laser beams that the components of light with different polarizations will in general have different reflection coefficients, it follows that these differing components will also have their phases changed by differing amounts. This implies that if light is assumed to be plane polarized when incident on a conducting

reflector, it will in general become elliptically polarized after reflection (22). Any further reflection will then change the polarization again, etc.

Now a receiver with circular cross section, such as a cone, will present a normal to the surface in all directions. Hence it will average the polarization of the beam within the cone at each reflection, making the coefficient of reflection independent of polarization. The coefficient will be the same for each reflection.

Receivers which do not present circular cross sections will not perform this averaging. Hence each reflection with a given wedge will have a different coefficient which depends on the state of polarization of the beam which is altered by each of the previous reflections. It is then difficult to predict the total attenuation of the beam within each wedge formed by receivers such as a razor blade stack.

All studies of the absorption by the walls of a receiver assume that those walls are smooth. In many commercial instruments, however, the cones are produced by welding, riveting, butting, or otherwise mechanically joining a sheet of metal to form the cone. The site of the joint will be a perturbation on the reflectivity of the cone and should be taken into account in calibrating the instrument. In addition, the surface of most metals is altered in time by oxidation or contamination, which will affect the reflectivity. Stainless steel razor blades appear to have advantages over cones in terms of design simplicity and surface resistance to chemical and physical damage.

Other devices, such as the Westinghouse radiometer RN-1, measure the change in resistivity of a metal wire as it is heated. The incident energy is thus determined. The method is acceptable, provided the temperature of the wire at all times exceeds the Debye temperature of the metal used in the wire. For copper the Debye temperature is 42°C, or 108° F (7). Above this temperature the resistivity of metals increases linearly with temperature (8). The wire can then be considered as many small segments connected in series, each with its own resistance dependent on the temperature of that segment. The sum of these resistances comprise the total resistance of the wire. Under these conditions, the heat distribution in the wire is not important; the total heat in the wire will determine the resistance. This principle insures that the rapidity of data output is limited solely by the circuitry involved. The errors due to heat loss occurring while the heat is evenly distributed are minimized. These instruments however, are not exempt from problems of reflection. The Westinghouse brochure on the RN-1, indicates that a correction must be made for reflected radiation which depends on both wavelength and polarization.

The majority of systems which employ devices which change the energy form from radiant to thermal use thermocouples or thermopiles as a sensing element. These thermocouples employ the thermoelectric effect (9), which is in reality three separate effects. The three effects are the Seebeck effect, the Peltier effect, and the Thomson effect.

In 1823, T.J. Seebeck discovered that if a circuit is formed consisting of two dissimilar metalic conductors, and if one of the junctions of the circuit is at one temperature while the other junction is at a higher

temperature, a current will flow in the circuit. The current continues to flow as long as the two junctions are at differing temperatures. The electromotive force producing this current is called the Seebeck Thermal EMF.

In 1834, M. Peltier discovered that if a current flows across the junction of two metals it gives rise to an absorption or liberation of heat (9). If the current flows across the junction in one direction heat is absorbed, while if the current flows in the other direction heat is liberated. If the current flows in the same direction as the current produced by the Seebeck effect at the hot junction in a thermoelectric circuit of two metals, heat is absorbed, while at the cold junction heat is liberated. The heat liberated or absorbed is proportional to the quantity of electricity which crosses the junction. The amount of heat liberated or absorbed when one coulomb of electricity crosses the junction is called the Peltier effect at the temperature of the junction. Expressing the heat in joules, it can be shown that the magnitude of the Peltier effect is given by the product of the absolute temperature of the junction and the rate of energy change of the thermal EMF of the junction at that temperature of the junction when the only current across it is that due to the thermal EMF (9).*

The Thomson effect was discovered by W. Thomson in 1847 from observations of thermocouples. He noted that if the only reversible thermal effects in the circuit were the Peltier effects at the junctions, the EMF around the circuit whose cold junction is kept at constant temperature should be

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* It is assumed that each junction of the thermocouple has small mass with respect to its temperature source.

proportional to the difference between the temperatures of the hot and cold junctions. This was contrary to experience. Thomson therefore looked for reversible heat effects when an electric current flows through a conductor in which there is a temperature gradient. He studied a copper wire whose temperature varied from point to point. When a current flowed along the wire such that at some point the current and heat flows were in the same direction, i.e. the current flowed from hot places to cold, heat was liberated. When the current and heat flows were in opposite directions, heat was absorbed. This effect produced by a current flowing along a conductor in which there is a temperature gradient is called the Thomson effect. This effect differs from the Peltier effect in that it occurs in a homogeneous conductor rather than at the junction of two dissimilar conductors. This effect has less influence on the temperature of the conductor than the Peltier effect has on the junction.* The Thomson effect can be detected experimentally only by the use of large currents and sensitive devices for measuring differences in temperature.

Thus the EMF observed by Seebeck, which is the basis of thermo-electric thermometry, is the algebraic sum of the Peltier EMF at the junctions and the two Thomson EMF's in the two dissimilar wires. There is therefore not a simple relationship between the temperature and the EMF observed. The relationship is certainly never strictly linear. The reason the EMF versus temperature curves are not linear, is that the Thomson EMF in one metal of the thermocouple circuit is not in general equal to the Thomson EMF in the other metal for all temperatures. However, for

* In all cases the Joulian heat loss has been neglected.

all thermocouples there is a range where the curve may be approximated by a straight line. The EMF versus temperature curves of thermocouples are tabulated (10). These tables are strictly empirical. The dependence of EMF on temperature can not yet be predicted with certainty for any given thermocouple.

The effect of high peak power levels on the reflective property of metals and on the energy measurement system requires further consideration. Whether the various devices remain linear under high peak power impacts requires further study. Daly (2) presumes that the conical collecting apparatus remains linear to the point of visible damage to the cone. Measurement of low energy levels can be attempted by integration of the output from photosensitive devices.

Several methods employing photomultiplier tubes as detectors of laser energy output have been reported (2,11, 12). Before individual methods are discussed, those characteristics of photomultiplier tubes which will affect all experiments will be noted.

Photomultiplier tubes consist of an envelope with a transparent section called a window, within which is a photocathode and a high gain, wide band current amplifier. The photocathode consists of a photosensitive material deposited on a carrier.

Photocathodes are produced in two ways. Those which emit electrons on the opposite side from which light is incident are called semi-transparent. This type is used in end-window tubes. Those which emit electrons on the same side as the light impinges are called opaque. These generally are

used in side window tubes. The semi-transparent photocathodes are produced by coating the inside of the window with the photosensitive material while in the opaque photocathodes the photoemitter is coated on an opaque metalic base.

Although the opaque photocathodes are simpler to make and are more sensitive, semi-transparent photocathodes are more generally employed due to their many design features (12).

When light is incident on a photomultiplier tube it must be transmitted through the window and then be absorbed by the photocathode. Both processes will give rise to a reflection which will in general be a function of the wavelength of the light and possibly the polarization(1).

From the familiar Einstein Photoelectric equation:

$$T = hV - \emptyset$$

where T is the kinetic energy of the emitted electron,

H is Planck's constant (6.624×10^{-34} j. sec.),

V is the frequency of the incident radiation,

$\emptyset$ is the work function of the photocathode,

it is evident that below a frequency V_o such that

$$hV_o - \emptyset \leq 0$$

there will be no emission of photoelectrons unless low probability events such as multiple photon absorption or electron tunneling take place. V_o corresponds to a wavelength λ_o through the equation:

$$v\lambda = C$$

where C is the velocity of light. Thus there is a cutoff of the photoemission above a wavelength λ_0 unless a low probability event occurs.

Finally, the efficiency of interaction of the incident radiation with the photocathode will vary as a function of wavelength (13). The combination of these three effects yeild a spectral response curve for each photomultiplier tube. The response curves of various types of photomultiplier tubes can be found in phototube manuals (13,14,15), and are usually supplied to the purchaser of a photomultiplier tube.

In many cases of interest, such as pulsed and Q-spoiled lasers, the radiant energy output is of sufficiently high density to destroy the phototube. In these cases, filters (11), diffusers (2,12) and beam splitters (16) have been employed to reduce the energy density before the beam is sampled.

Filters yield unreliable data since they can be calibrated only for low energy incidence and do not take into consideration either the spatial inhomogeneity of the beam, or the existence of hot and cold spots in the detector (12) which may arise due to an inhomogeneity in either the photomultiplier tube window or the photocathode.

Diffusers are employed to reduce the energy density incident on the phototube in the following way. The laser is fired at the diffuser which scatters the radiation in all directions. A uniformly diffusing surface is defined as "one for which the luminous intensity per unit area in any

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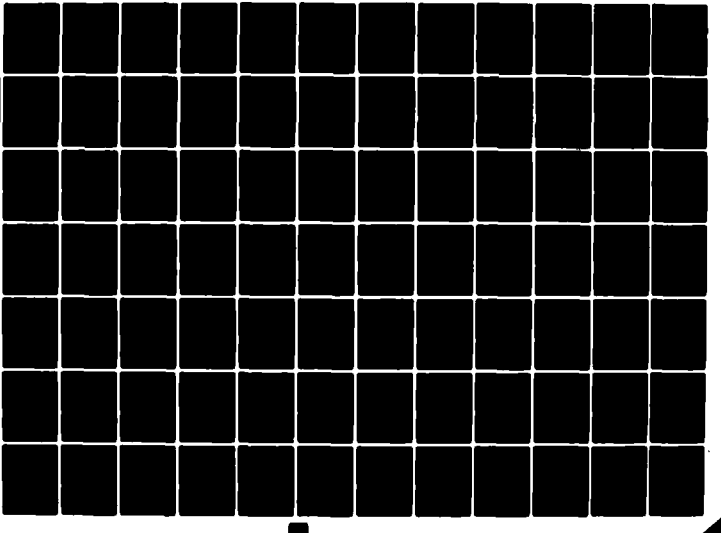


direction varies as the cosine of the angle between that direction and the normal to the surface, so that it appears equally bright whatever be the direction from which it is viewed" (19). A surface which exhibits this property is sometimes called a Lambert surface.

Daly (2) describes a procedure using as a diffuser, a "MgO block with reflectivity ρ." He states that if the phototube has aperture A, and is a distance D away from the diffuser, the fraction L of the original emitted power intercepted by the phototube aperture is given by

$$L = \frac{A \rho}{D^2 \pi}$$

This formula is applicable providing ρ is known as a function of both the position of the phototube and the wavelength of the light incident on the diffuser. However, determination of ρ may be difficult, particularly at high peak power density levels. Extrapolation from data obtained at low power levels is not necessarily justified. In addition, no correction has been made for the specular reflections which have been shown to arise whenever light is incident on a rough surface A relation between surface roughness and specular reflectance at normal incidence has been found (17) which is dependent on wavelength.

Bernal (18) has shown that single MgO crystals become strongly absorbing when exposed to the focused beam of a normal pulsed laser. The strong absorption coincides with the vaporization of the crystal by the beam.

Leite and Porto (12) use a $BaSO_4$ coating on glass which they measured to have a 98.5% reflectivity and to be a nearly perfect Lambert reflector. They do not indicate the wavelength at which the reflectivity of 98.5% was obtained, nor whether the value is wavelength independant. Most reflectors are wavelength dependent (20).

In devices called beam splitters, the usual technique is to insert a transparent medium at an angle to the direction of the beam, thereby partially reflecting and partially transmitting the beam. The amount reflected can be varied by changing the angle of the reflector with respect to the beam. Since this device introduces another reflection into the system, its effect is identical to those discussed elsewhere in this paper (1).

Summary of Thermocouples

Thermocouples employ the Seebeck effect which, if joulian heat loss is neglected, can be considered the sum of the Peltier and Thomson effects.

The Peltier effect is a reversible effect. The process involved is the production of heat flow into or out of a junction between two different metallic conductors when a current passes through the junction.

The Thomson effect is also reversible. It is a heat flow into or out of a homogeneous conductor in which there is a temperature gradient. If the current is in the same direction as the heat flow in the wire, heat is liberated.

Thermocouples are nonlinear devices because the Thomson EMF in each of the components of the device varied differently with temperature gradient.

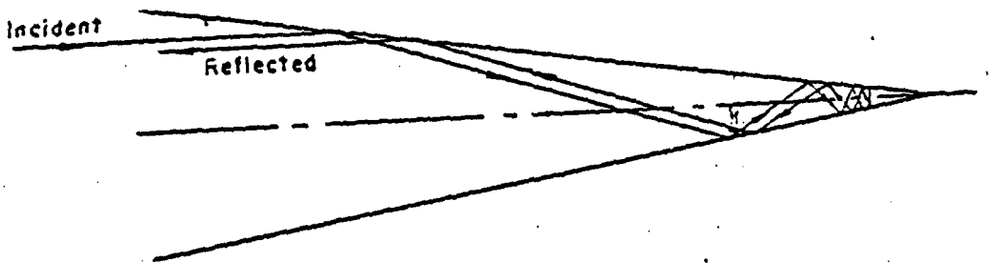


Fig. 1. Mendenhall wedge effect.

The above diagram of a specularly reflecting surface shows 13 reflections. If the cone reflects 90% of the light at each point, then the reflected ray has $(.9)^{13}$ or about 25% of the energy of the incident ray.

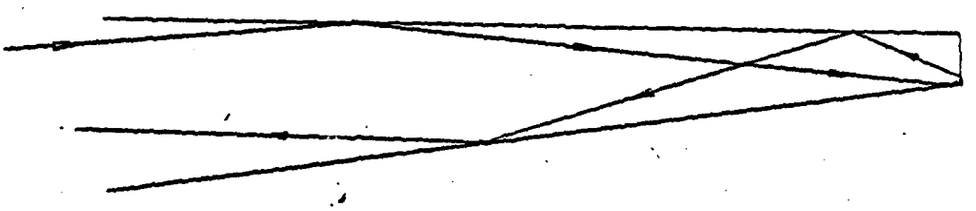


Fig. 2. Truncated cone.

Flat end interferes with wedge effect, thereby reducing the number of reflections.

References

1. See section on polarization of laser beams

2. Daly, R., "Measuring Laser Performance," Microwaves, January 1964.

3. Eisenman, W. et al, "Black Radiation Detector," JOSA, 53:6, June 1963.

4. Gouffé, A., Rev. Optique, 24(1), 1945

5. Ackerman, J.A , "Laser Energy Measuring Device," Applied Optics, 3(24), 1964.

6. Dimeff, J., and Neel, C.P., NASA Tech. Brief, 63-100-04, April 1964.

7. Dekker, A.J., Solid State Physics,(New Jersey:Prentice Hall, 1962) p. 44

8. Ibid, p. 225.

9. Dike, P.H., Thermoelectric Thermometry,(Phila, Pa.: Leeds & Northrup Co.,), p. 1.

10. Handbook of Chemistry and Physics, College Edition, 45th Edition, (Cleveland, Ohio: Chemical Rubber Pub. Co., 1964-65), p. E47.

11. Glick, A.L., "A Method for Calibration of Laser Energy Output," Proc. IRE, 50:1835, Aug. 1962

12. Leite, R.C.C., and Porto, S.P.S., "A Simple Method for Calibration of Ruby Laser Output," Proc. IEEE, 51:606, 1963.

13. "Photomultipliers for Scintillation Counting," distributed by Philips Electron Tube Division.

14. "DuMont Multiplier Phototubes," Dumont Laboratories, Div. of Fairchild Camera & Inst. Corp., Clifton, N.J., March 1963, p.2.

15. "R.C.A. Phototubes and Photocells," technical manual PT-60, Radio Corp. of America, Electronic components and devices, Lancaster , Pa., 1963.

16. Commercial devices such as the optical calorimeter model 600 by Optics Technology Inc.

17. Bennet, H.E., and Portens, J.O., "Relation Between Surface Roughness and Specular Reflectance at Normal Incidence," JOSA, 51:123,1961.

18. Bernal, E.G., "Absorption of Laser R diation by Transparent Crystalline Solids," abstract presented at the 1965 Spring Meeting, Optical Society of Amer., Dallas, Texas, Mar. 31-Apr.2, 1965,(paper TA16).

19. Walsh, J.W.T., Photometry (London: Constable & Co., LTD, 1953) p. 137.

20. Ibid. Appendix VII.

21. Ditchburn, R.W., Light (New York: Interscience Publ., Inc. 1963) p. 551.

22. Ibid. p. 555.

Hazards Due to Light Scattering

In this section of the report the hazards to be expected due to scattered light will be indicated. The problem (of scattered light) will be considered in three separate sections. First, the scattering properties of the atmosphere will be considered under different atmospheric conditions. Second, some typical examples to diffuse reflectance (backscatter from targets) that may be encountered in the laboratory will be cited. Finally, the "hazard regions" associated with conditions considered in sections One and Two will be outlined. Since laser communications and experimentation will be carried out at a variety of power levels, pulse widths and beam geometries, it is not possible to specify a "hazards region" without also specifying the characteristics of the associated laser. Consequently in Section Three the power density of scattered radiation will be expressed relative to the power output of the laser. The resulting ratio, $\frac{I\ sca}{I_o}$, will then be a function only of the position of the observer and the properties of scattering media under consideration. In this way safe distances associated with any specific laser output characteristics and scattering condition can be determined utilizing the hazard power levels presented in other sections of this report. Some examples in which all the variables have been fixed (re laser characteristics and scattering properties of the media or target) will be given to illustrate how the information presented in section Three is utilized.

INTRODUCTION

At the usual low power levels the degree to which a differential layer of a medium diminishes a beam of radiation is determined by the extinction coefficient of the medium. Extinction may be due to scattering or absorption or both, so that in general under the above condition

$$\frac{I\ (z+dz)}{I\ (z)} = \frac{dI}{dz} = -I\alpha_{ext} = -I\ (\alpha_{sca}+\alpha_{abs})$$

where α_{abs}, α_{sca}, and α_{ext} are the absorption, scattering and extinction coefficients respectively, and I is the radiation intensity. The scattering coefficient (turbidity) is a measure of the radiation which passes undiverted from one layer of the medium to the next. The absorption coefficient indicates the

radiation absorbed in each layer.

Electromagnetic radiation is scattered whenever it passes through an imhomogeneous medium. The two mechanisms by which light is scattered are refraction and diffraction. Refraction occurs when radiation is diverted crossing a boundary between two dissimilar optical media. Diffraction is an interference phenomena which is associated solely with the geometry of the scatterers (1a).

The extent and spatial distribution of the scattered radiation depends on the characteristics of the radiation and the inherent properties of the inhomogenieties and their number density. If the beam diameter is small compared with other dimensions of the systems the intensity at any particular cross-section, a distance z into the medium, along the direction of propagation will be

$$I_o \, e^{-\alpha_{ext} z}$$

where I_o is the incident power density. If the beam width is increased, intensity will no longer be constant at any given cross-section.

When the inhomogenieties are particles the total radiation scattered to a given point will be the sum of the radiation scattered from all particles plus the undisturbed part of the incident beam at that point. In the limiting case in which particle separation is large enough so the inter-particle radiation is negligible (single scattering) the scattering function associated with all particles will be identical and the radiation scattered to any point may be evaluated by a simple volume integral (1), such as that in Equation 1 and Figure 1.

$$I = \int_v I_o \, f(R, \theta, \phi) \, dV_e$$

$$= \int_v I_o \, f(R_o, \theta_o, \phi_o, R_e, \theta_e, \phi_e) \, dV_e$$

where V is the total volume containing scatters, dV_e is a small element of the total volume which scatters the light incident upon it according to $f(R, \theta, \phi)$ and the variables (R,θ,ϕ), $(R_o,\theta_o\phi_o)$ and (R_e,θ_e,ϕ_e) are the position of a scattering element with respect to an observer, the position of the observer with respect to the origin, and the position of the scattering element with respect to the origin.

If interparticle radiation is significant (multiple scattering) the scattering function associated with an individual particle is dependent on the position

of that particle and the distribution of particles in the system (2). Problems of this nature can in general be numerically evaluated only with the aid of severe approximation

Due to the complex structure associated with biological media, their turbidities are very high. In the liver, for example, the scattering coefficient is an order of magnitude greater than the absorption coefficient (3). Consequently, when lasers are incorporated in clinical treatment, it should be recognized that radiation scattered to points outside the path of the beam may be of considerable intensity.

Atmospheric Transmission

Before considering atmospheric scattering it is necessary to consider atmospheric absorption since radiation absorption by the atmosphere limits the useful frequency range for communications purposes. The absorption coefficient, like the scattering coefficient will depend upon the number density of various atmospheric constituents and the wavelength of the radiation for normal atmospheric haze. The frequency ranges in which absorption is high under these conditions are in the ultraviolet and infrared but not in the visible, except for a few narrow bands.

The absorption coefficient for atmospheric components as a function of wavelength has been determined by Thompson et al. (4) for the range between 1850 Å and 4000 Å (ultraviolet). His results are reproduced in Figures 2-9 and the pressure at which the tests were carried out in Table I. The results were obtained with a double beam spectrophotometer and a test cell with a 10 cm path length. The normalization procedure for tests run with a particular gas at different pressures was not indicated (see Table I, in particular data concerning NH_3).

The absorption coefficient of gases is highly dependent upon pressure. Consequently the data of Figures 2-9 do not indicate the relative absorption coefficient of the atmospheric fractions as they are found in nature since the gases are not present in equal amounts and certainly not at the concentration indicated in the report. However, the high absorption characteristics of all constituents below 2000 Å would limit transmission to longer wavelengths in the lower atmosphere. At high altitudes (20 Km) the absorption of ozone in the middle ultraviolet (2000 Å to 3200 Å) limits the useful range to above 3200 Å (2).

Atmospheric absorption in the infrared is determined to a great extent by the behavior of two atmospheric components H_2O and CO_2 (6). A detailed theoretical evaluation of the absorption spectra of these molecules in the infrared range averaged over frequency intervals of 2.5 cm^{-1} for three temperatures between 200 and 300°K, at seven pressures from .01 to 1 atm. has been presented in two Air Force reports (7,8). The analyses were based upon the quasi random model for molecular band absorptance (9). Due to its complexity, a discussion of the model will not be given here. A comparison of the spectra analytically determined in the report by Wyatt, Stull and Pluss with experimental results of Burch et al. (10) was given in subsequent articles (11,12). Graphs from these articles have been reproduced in Figures 10 and 11, along with the atmospheric conditions to which they correspond. The high correlation between the two sets of data tends to indicate the validity of the model used for analytical evaluation over the ranges tested. Since considerable detail concerning atmospheric absorption at infrared wavelengths has been given both analytically and experimentally by Wyatt, Stull and Pluss, and Burch et al., respectively, only a brief summary of the characteristics will be given here.

Unlike the ultraviolet region, the infrared contains many windows (spectral bands of transmittance). Consequently it is difficult to define a transmission cutoff point at long wavelengths. Like the ultraviolet there is a strong dependence of absorption upon altitude. This is apparent when one considers the atmospheric absorption spectra for beams of solar radiation reaching different altitudes. Two such spectra are shown in Figure 12 (by Goody (13)) for solar radiation reaching the ground level and that reaching 11 Km. The specific atmospheric conditions under which the data was taken is not indicated in the article, since the spectra presented were intended by the author to illustrate relative absorption at different altitudes under "typical" conditions. The continuum in Figure 10 was interpreted by the author as the result of the scattering continuum which was not distinguishable from directly transmitted light.

In view of the data considered, it was concluded that vertical atmospheric transmission of laser radiation for communications purposes is limited in general to wavelengths between 0.3 and 1.5μ. For horizontal transmissions at altitudes where ozone content is negligible, (less than 15 Km (14)) the near ultraviolet region (.2 to 3μ) may also be utilized. Since the useful range will depend upon atmospheric conditions, transmission bands will vary with geographical location and season. However, transmission through the infrared windows may be

important... Consequently the transmission range presented here should be interpreted as corresponding to an average situation.

The scattering of light by the atmosphere has received considerable attention in past literature with emphasis placed primarily on extinction (15,16,17) rather than angular distribution. Although the extent and manner in which light will be scattered by individual atmospheric particles can be predicted theoretically (18, 19) the problem of aggregate scattering, except under special conditions, can only approximately be represented analytically. In fact, when the light scattering properties of the atmosphere have been studied (20,21,22,23) it has been found that the angular distribution of scattered light did not correspond to any simple distribution (in both size and type) of particles. The noted characteristics appeared to be the additive effects of Lambertian scatterers (dust) large dielectric spheres (water droplets) and Rayleigh scatterers (gas molecules). The extent and distribution of scattered light is dependent on the concentration of these particles as well as on their size distribution in the case of dust and water droplets. Assuming single, incoherent scattering, the scattering coefficient per unit volume, α_{sca}, will be the coefficient per particle times the number of particles per unit volume, summed over all particles in the volume or

$$\alpha_{sca} = \int_I N(i)\alpha(i)\, di$$

Similarly, assuming incoherent (random phase) scattering the angular distribution of scattered light is

$$S(\theta) = \int_I N(i)S(i,\theta)\, di$$

where $S(i,\theta)$ is the distribution of scattered light associated with the ith particle. An evaluation similar to that described above was completed by Deirmendjian (24). The evaluation was based on an empirical expression proposed by Khrgian and Mazin (25,26) which described the size distribution of particles found under various atmospheric conditions, with the characteristics of particles of a specific size taken from Mie theory (27). Some of the results of this evaluation are given in Figures 14-17.

Dust particles scatter light primarily to the second and third quadrants (backscatter) water droplets to the first and fourth quadrants (forward scatter) and molecules equally in all quadrants (28). Consequently the scattering of

light near urban centers will be primarily backscatter while maritime atmospheres will exhibit forward scattering.

The power density of radiation at the retina of an observer is determined by the amount of light scattered from specific points in the beam due to the imaging properties of the eye. Consequently the scattering properties of a small volume of scatterers (Figure 18) within the beam of radiation, must be considered.

The angular distribution of light scattered by such a volume irradiated by a Xenon flashlamp beam of small divergence has been studied by Reeger and Siedetopf (29). The light emerging from this volume was measured with a directional photometer. The field of view of the photometer determined the dimensions of the observed scattering volume. The data associated with this study was taken in normal atmospheric haze (of low turbulence, turbidity, and dust content). Since $I=I_o e^{-\alpha_{sca} z} f(\theta)$ is the intensity per steradian it can be concluded that the intensity per square centimeter is

$$I(\theta) = I_o e^{-\alpha_{sca} z} f(\theta) \left(\frac{0.01}{R}\right)^2 \text{ watts/cm}^2$$

where I_o = the intensity of radiation at the source

R = the radial distance of the observer to the scattering volume in meters

z = the distance from the source to the scattering volume

θ = angle measured with reference to the forward direction

α_{sca} = the turbidity of the atmosphere

If $R \gg 0.01$ the rays will be almost parallel. As a worst case, assuming a dark adapted eye the focused spot size at the retina will be 16μ (30) and

$$I_{retina} = \frac{I_o e^{-\alpha_{sca} z} f(\theta)}{R^2} \quad 10^2 \text{ watts/cm}^2$$

where R is in meters.

Since α_{sca} in general ranges from 0.1 to 0.05 Km^{-1} (31), for $z \leq 1$ Km

$$I_{retina} = \frac{I_o f(\theta)}{R^2} \quad 10^2 \text{ watts/cm}^2$$

Figure 20 is a plot of $I_{retina} = 10^{-3} I_o$ with $f(\theta)$ based on the data of Reeges and Siedentopf. This Figure represents the integrated scattering pattern of the light at all wavelengths transmitted by the flashlamp. Since very little

change is observed in extinction coefficient as one traverses the visible range, this pattern and those at specific visible wavelengths would in general be similar. However, at specific wavelengths, resonant conditions dependent on the size and number density of atmospheric particles may alter the scattering pattern.

At wavelengths where the cornea has a significant absorption coefficient (UV and IR) most of the radiation incident on the eye will be absorbed near the surface. Consequently the total light scattered to a point must be considered rather than the light scattered from a specific part of the beam. If analysis is limited to distances from the beam, R, from which $\alpha_{sca}R<<1$

$$\frac{e^{-\alpha_{sca}R}}{R^2} \approx \frac{1}{R^2}$$

The scattered light intensity absorbed by the cornea at the observation point, R , θ (see Figure 19) due to the entire beam will then be

$$I = \frac{I_o\, e^{-\alpha_{sca}z}\, f(\theta)\, G(\theta)}{R^2}\, dz \qquad (1)$$

where $f(\theta)$ is the atmospheric scattering function (as described on page 6 of this report) and $G(\theta)$ is the angular dependence of absorption of the cornea.

Utilizing a stepwise approximation for $G(\theta)$ and $f(\theta)$

$$I \simeq \sum_{n=m}^{N} \int_{\Delta n} \frac{I_o\,(1-\alpha_{sca}z_n)\, f(\theta)_n\, G_n(\theta)_n}{X_o^2 + (z_n - z_o)^2}$$

$$\simeq \sum_{n=m}^{N} \left[\frac{\Delta\theta}{X_o} - \frac{\alpha_{sca}}{2} \ln\left(\frac{\csc^2 \theta_{n+1}}{\csc^2 \theta_n} \right) \right] F_n A_n$$

where $\theta_m = \arctan \frac{X_o}{z_o}$ (corresponding to z=o).

Due to insufficient data concerning both $f(\theta)$ and $G(\theta)$ at ultraviolet and infrared wavelengths computer evaluation of $I(\theta)$ was accomplished assuming

(1) $G(\theta) = 1$

(2) $f(\theta)$ = the same in the near IR, visible, and near UV

The results of the evaluation are shown in Figure 21. It is emphasized that Figure 21 is meant only as an indication of the distribution of scattered light. A more detailed evaluation would be warranted only if more information concerning $f(\theta)$ and $G(\theta)$ were available.

In fog, clouds, and other optically dense media turbidities are of the order of 15 Km^{-1} (32). It can be concluded that for turbidities this high a collimated beam of light is diminished to roughly 0.2 of its original intensity in 100 m. The distribution of the scattered energy cannot readily be determined analytically due to multiple and directed scattering. Search of the literature to date has provided little information concerning distribution of the light scattered from a beam in a highly turbid media. Consequently the following model has been postulated in order to determine the distribution of scattered light one may expect under highly turbid conditions.

If it is assumed that the intensity of the beam a distance, z, into the turbid media is

$$W = W_o e^{-\alpha_{sca} z} \text{ watts}$$

The power lost in the length Δz will then be

$$dW = \frac{dW}{dz}\Delta z = W_o \alpha_{sca} e^{-\alpha_{sca} z} \Delta z$$

Under the assumption of radial propagation of scattered light from the center line of the beam and an exponential decay in the radial direction, a

$$I = \frac{W_o \alpha_{sca} e^{-\alpha_{sca}(z+a)}}{2\pi a} 10^{-2} \text{ watts/cm}^2$$

where a is in meters (see Figure 19).

Assuming a 16μ diameter spot on the retina (33) the power density at the retina is

$$I_{retina} = \frac{W_o \alpha_{sca} e^{-\alpha_{sca}(z+a)}}{2\pi a} \times 10^4 \text{ watts/cm}^2$$

(Assuming an ocular gain of 10^6)

For $\alpha_{sca} = 15 \text{ Km}^{-1}$

$$I_{retina} = \frac{W_o \exp(-1.5 \times 10^{-2}(z+a))}{a} \times 2.4 \times 10^{-1} \text{ watts/cm}^2$$

The scattering properties of snow have been studied experimentally by Hogg (34). A helium neon laser, 0.63μ, was transmitted through a cassegrainian telescope over a 2.6 Km path length. The intensity of the beam was measured at various angles while the path length was kept constant. Data for various turbidities is shown in Figure 22. It is noted at this point that, in general, the scattered radiation may not be represented by

$$I = \frac{I_o f(\theta_o)}{R_o^2}$$

where θ_o and R_o are the coordinates of the observer with respect to the source. Consequently, experimental data such as reported by Hogg (35), concerned with the angular dependance of scattered radiation intensity in turbid media, give information only about the data points and may not be extrapolated to determine light intensity at other points.

The turbidity of the atmosphere decreases as wavelength increases in the visible and infrared regions (36). This is due to the fact that the scattering cross-section of water droplets, large compared to wavelength decreases with increasing wavelength. However, the decrease is generally less than an order of magnitude as one traverses the entire visible spectrum and into the infrared region. (This is in contrast to the λ^{-4} dependence found in the microwave region). Consequently, one may conclude that the atmospheric scattering patterns, for all wavelengths within the visible range, will be similar.

In addition to particulate scattering, spatial variations in atmospheric conditions will also result in the scattering of radiation. Edwards and Steen (37) have shown that a constant level optical signal transmitted through a turbulent atmosphere will also be randomly modulated. The signal variation was designated by a modulation index given by

$$M^2 = \frac{\overline{D^2(t)}}{\overline{I(t)}^2}$$

where $D(t)$ = deviation from the average

$\overline{I(t)}$ = average signal

The data was taken in normal atmospheric haze with relatively low turbulence (although no exact figures concerning turbulence were given). Observed modulation indices were reported to vary according to

$$M^2 = \text{constant}/D^{\alpha}$$

where D = viewing aperture diameter, and α = constant nearly equal to unity. This is probably due to the fact that spatial variations tend to average out as the beam cross-section is increased.

Modulation indices as high as $M^2 = 0.4$ were observed with signals of a 3 cm^2 cross-section. The data showed random seasonal dependence although the specific effects of variable ambient atmospheric conditions were not indicated. The significant effect of turbulence in a medium of low ambient turbidity suggests that turbulence in optically dense media will play a significant role in atmospheric scattering. Since the distribution will be random and time varying, statistical analysis will be necessary in order to determine probable scattered radiation intensities.

Scattering of Light by Targets

Probably a more eminent hazard is diffuse reflection and scatter from targets. It has been shown (38) that the diffuse reflectivity at angles within 15° from the normal, of a surface with the following properties

(1) RMS roughness, $\hat{\Omega}$, (RMS deviation from flatness) is small compared to wavelength.

(2) Distribution of heights of the surface is gaussian about the mean.

is that of Equation 2:

$$R_d = \frac{R_o 2^5 \pi^4}{M^2} \left(\frac{\Omega}{\lambda}\right)^4 \left(\frac{S}{R}\right)^2 \qquad (2)$$

where M = RMS slope contained in the surface profile, R_o is the total normal reflectance, S = observation arc length, and R = radial distance to the center of the surface. For a surface described by $\frac{\Omega}{\lambda} = 0.1$, m = 0.01, and $R_d = 10^{-3} cm^2$ at a radial distance of 1m.

The optical characteristics of the target are time varying at high power densities. This is partly related to volatilization and creation of a plasma cloud or plume over the surface. The diffuse reflectance described above will consequently be an initial value. Estimated electron densities of plumes range from 10^{15} electrons/cm^3 for dielectrics, to 10^{23} electrons/cm^3 for metal surfaces. The amount of energy reflected by the plasma is dependent upon its complex refractive index which is given by (39)

$$n = \left(e_o\left[1 - \frac{Ne^2}{m(v^2+w^2)} + i\frac{Ne^2}{m(v^2+w^2)}\right]\right)^{\frac{1}{2}}$$

where N = electron number density

e_o = permitivity of free space

m = electron mass

e = electron charge

v = plasma collision frequency

w = the frequency of the optical radiation

Initially if the plasma electron density is greater than $10^{20}/cm^3$, the index of refraction is a pure imaginary number ($\sqrt{-1}\,n$, where n is a real number) and the plasma is highly reflecting. As the plasma disperses, its electron density decreases until the refractive index becomes real and the plasma reflects little of the incident energy. When the plasma has dispersed to a point where the

electron density reaches a critical value, which is of the order of $10^{20}/cm^2$, it will again be highly reflecting. Assuming that the leading edge of a plume emerges from an irradiated surface is a spherical segment, the radiation will be reflected according to

$$I = I_o \tfrac{1}{2} \left(\frac{a}{R_o}\right)^2 \frac{1}{1 - \cos 2 \sin^{-1} \frac{a}{r}}$$

(see Figure 23) for angles equal to or less than $2 \sin^{-1} \frac{a}{r}$

where r = radius of curvature of the plume
a = radius of the incident beam
R_o = radial distance of the observer

Thus for a plume with a radius of curvature three times the radius of the beam at a distance, R_o, 1m from the event, the scattered intensity is roughly 10^{-4} the incident radiation.

At a distance of only 1m. from the source of scattered radiation one may not assume ocular focusing to a diffraction limited spot. The image diameter is given in this case by (40)

$$y = \frac{\arctan (X/D)}{F + D}$$

where X = object diameter
D = distance to the object
F = refractive power of the eye ($=60m^{-1}$) (41)

Assuming that the scattered radiation emerges from an area of 1 cm diameter, the image spot diameter at the retina is $y = 1.67 \times 10^{-2}$ cm. The corresponding image area is then 1.9×10^{-4} cm^2. Assuming that the pupil is dilated so that its area is 1 cm^2 the gain due to focusing of the eye in this case is roughly 5×10^3. The power density ($watt/cm^2$) at the retina will then be 5×10^{-1} the output power (watts) of the laser at 1 meter from target.

When the plume is totally reflecting, the radiation it scatters from a focused beam will be related to the incident power density in roughly the same way as in the unfocused case. The divergence of the scattered radiation may be either greater than or less than in the unfocused case depending on the focal length of the lense used, but the scattered radiation intensity will in general be of the same order of magnitude.

Safety Regions

The spatial dependence of scattered radiation (at visible wavelengths) under the condition of normal atmospheric haze presented above has been expressed in terms of a fixed scattering element and a variable observation point. In order to establish positions of safety the spacial distribution of scattered light must be expressed in relation to fixed observation points. The difference between these two approaches is illustrated in Figures 18 and 19

On page 6 of this report it was shown thatat visible wavelengths, the light power density at the retina is given by

$$I_{retina} = I_o \frac{f(\theta)}{R^2} \quad 10^2 \qquad (3)$$

Equation 3 may be expressed alternately by

$$I_{retina} \quad \frac{I_o \; f\,(\theta')}{a^2} \quad \sin^2 \theta' \times 10^2$$

θ' is the angle of the observation (see Figure 19). The radiation intensity of the retina will be tolerable when $I_{retina} < I_{hazard}$ or when:

$$\frac{I_{hazard}}{I_o} > \frac{f(\theta') \sin^2 \theta' \times 10^2}{a^2}$$

where I_{hazard} is the hazard level at the retina.

The function of $f(\theta) \sin^2 \theta \times 10^2$ is plotted in Figure 24 where $f(\theta)$ is taken from the data on atmospheric haze of Reeger and Siedentopf (42). The function has a maximum of 1.6×10^{-2} at approximately 12° which corresponds to the direction of observation at which the scattered light intensity is maximum. Assuming this value as a worst case, the minimum radial distance from the beam, under normal atmospheric conditions, at which an observer will be safe is

$$a = 1.26 \times 10^{-1} \sqrt{\frac{I_o}{I_{hazard}}}$$

Thus for a 10 megawatt/cm^2 laser pulse, of duration 1 millisecond, and a corresponding hazard level at the retina of 1 Kw/cm^2 the safety distance is roughly 12 meters.

The distribution of scattered light in highly turbid media ($\alpha_{sca} \doteq 15$ Km^{-1}) was given on page (in the section on atmospheric transmission) by

$$I_{retina} = 2.4 \times 10^{-1} \frac{W_o e^{-1.5 \times 10^{-2} (z+a)}}{a}$$

under the assumptions cited. The radial distance from the center line of the beam at which an observer will be safe is then found from the solution of a transcendental equation.

The radial distance, a, required for safety decreases exponentially with z, the direction along the center line of the beam. Maximum hazard occurs consequently at z = o where

$$\frac{W_o}{I_{hazard}} = a e^{+1.5 \times 10^{-2} a} \times 4.2$$

This equation is plotted in Figure 25 for $\frac{W_o}{I_{hazard}}$ ranging from 1.0 to 10^3. The

The safe radial distance, a, will then be given by

$$a = a_o e^{-\alpha_{sca} z}$$

where a_o=the safe radial distance at z=o, as read from Figure 25. Thus for a 1 megawatt pulse of duration 1 millisecond and the corresponding hazard level at the retina of 1 Kw/cm^2 the ratio $\frac{W_o}{I_{hazard}} = 10^3$ and the safe distance under highly turbid atmospheric conditions ($\alpha_{sca} = 15$ Km^{-1}) will have a maximum value of 76m at z=o and decay exponential as indicated. Until now beam divergence has not been discussed in this section. It is apparent that since radial safety distances, associated with scattering by normal atmospheric haze are small, at large values of z beam divergence will be the controlling factor. For small z, however, i.e. positions near the source, divergence does not result in appreciable beam spread, and the safety distance associated with scattering predominates. (A discussion of the effects of beam divergence has been presented in another section of this report).

Under highly turbid conditions light is scattered to a much greater extent than in normal haze. The effect may be thought of as beam broadening due to scattering. This broadening effect will be much greater than the broadening effect of inherent beam divergence. Consequently under highly turbid conditions beam divergence is not a significant factor.

At intermediate values of turbidity the beam divergence and scattering may not be considered separately since their effects are comparable. A more detailed investigation is required before safety distances may be established for conditions of intermediate turbidity.

As was illustrated above, the power density at the retina that one may expect due to scattered radiation in the laboratory could be of the same order of magnitude as the power output of the laser. Consequently a 100j laser could result in 12 j/cm^2 at the retina of an observer two meters from the target. This level is well above the damage threshold. If we relax the assumption that the plume is 100% reflecting and assume only

10% reflectance (lower electron density in the plasma) the scattered radiation remains above the damage threshold. Moreover since the lesion cross-section (10^{-4} cm^2) constitutes such a small percent of the total retina area, the observer may not be aware that damage has been done until several lesions have been produced. It is concluded that a laboratory in which laser research is carried out should be considered a critical area. An adequate warning system should be provided which will indicate when the laser is "active" and protective precaution taken during the firing.

The hazards associated with scattered ultraviolet and infrared radiation differ considerably from those connected with visible radiation. The total radiation from all points within the field of view must be considered addatively. Before this summation is carried out each component incident on the cornea must be weighted with the absorption coefficient characteristics of the angle of incidence of that component. It is concluded that due to the complexity of the problem, a more thorough investigation is required before a criteria for safety can be established concerning scattered ultraviolet and infrared radiation.

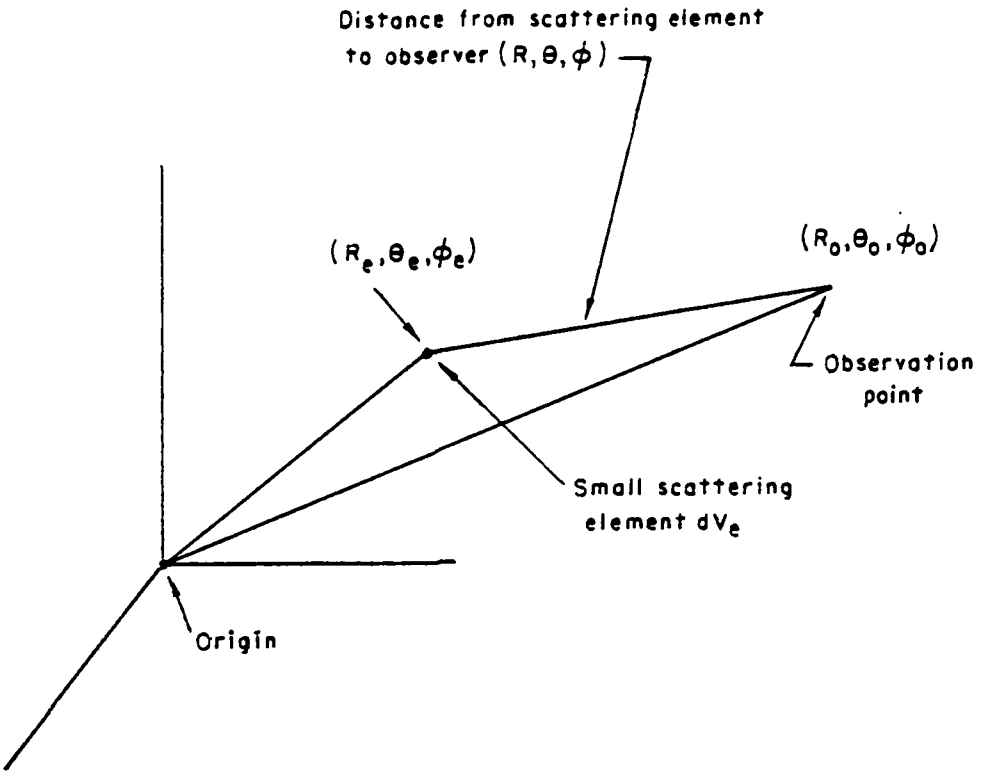


FIGURE 1 Geometry for Evaluation of Single Scattering

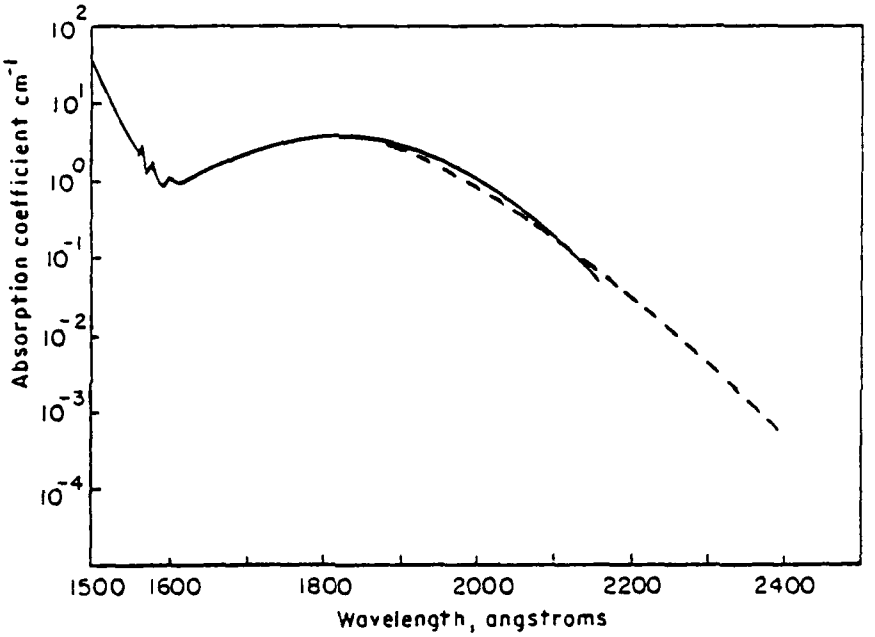


FIGURE 2 Absorption coefficient of N_2O as a function of wavelength.

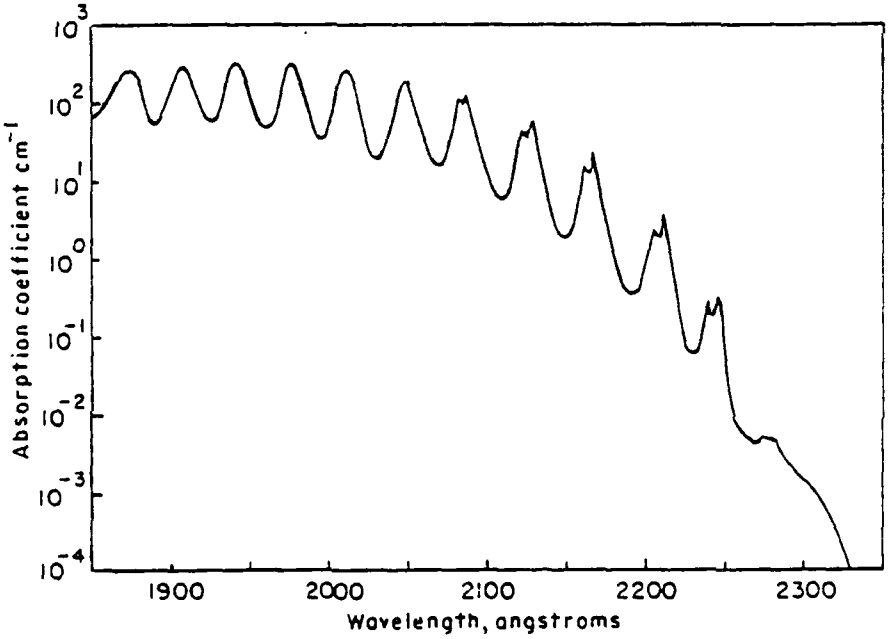


FIGURE 3 Absorption coefficient of NH_3 as a function of wavelength.

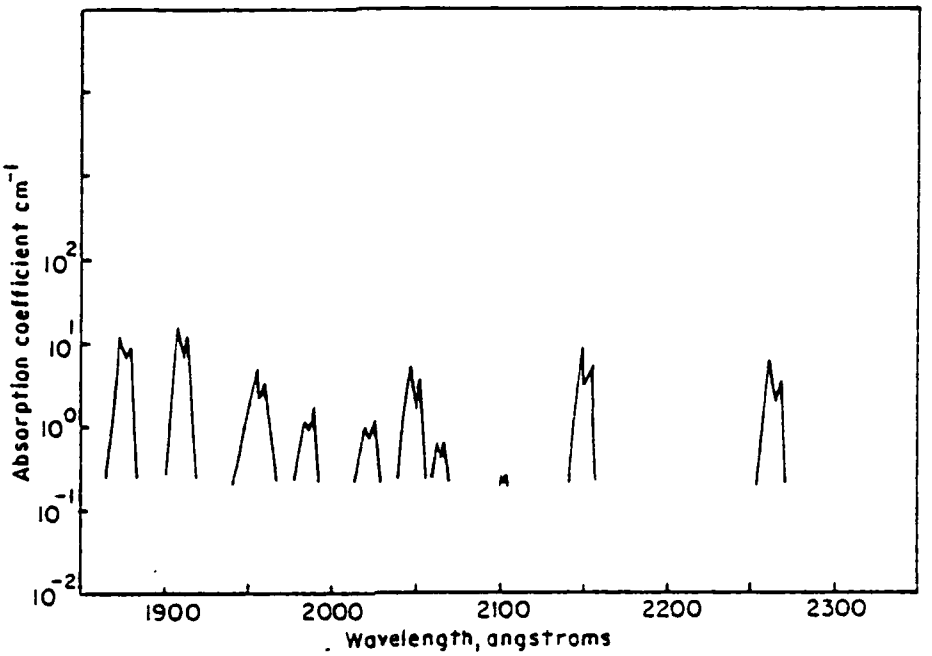


FIGURE 4 Absorption coefficient of NO as a function of wavelength.

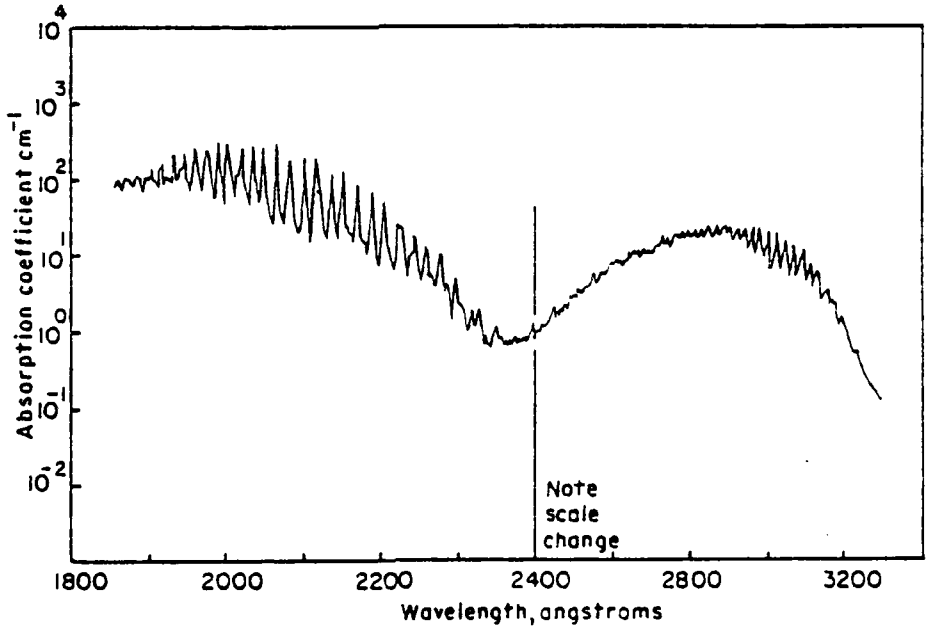


FIGURE 5 Absorption coefficient of SO_2 as a function of wavelength.

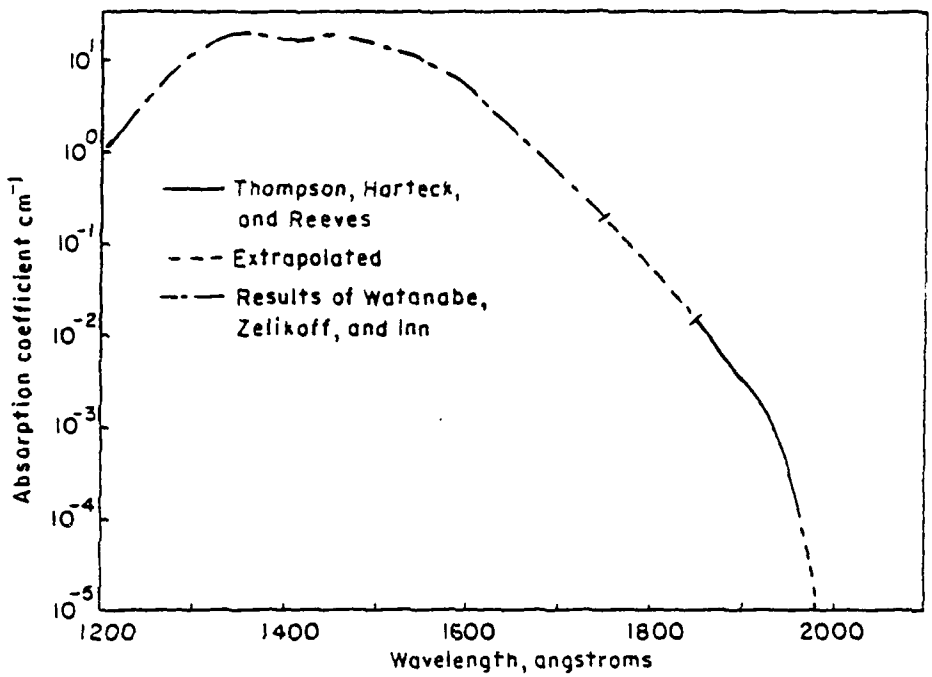


FIGURE 6 Absorption coefficient of CO_2 as a function of wavelength.

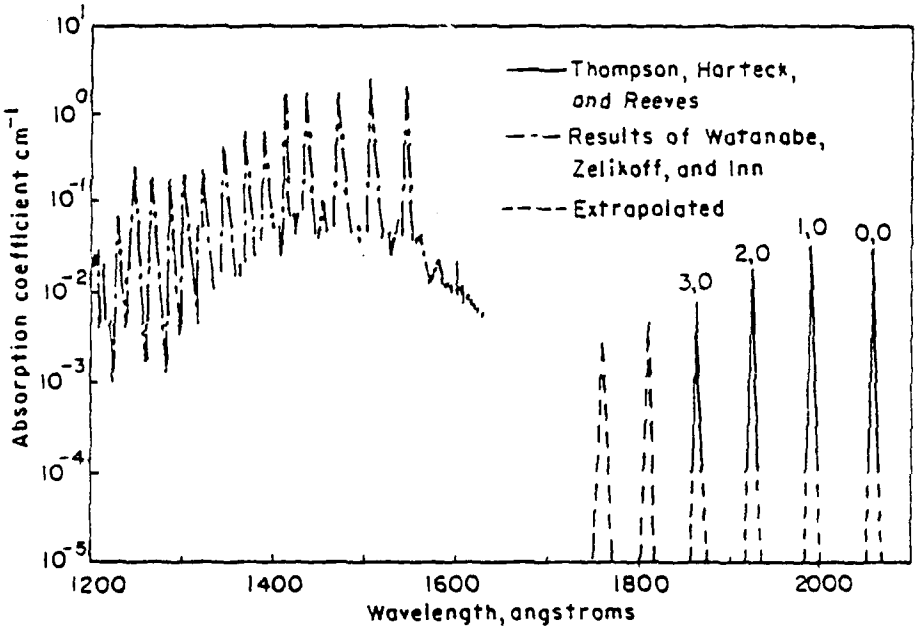


FIGURE 7 Absorption coefficient of CO as a function of wavelength.

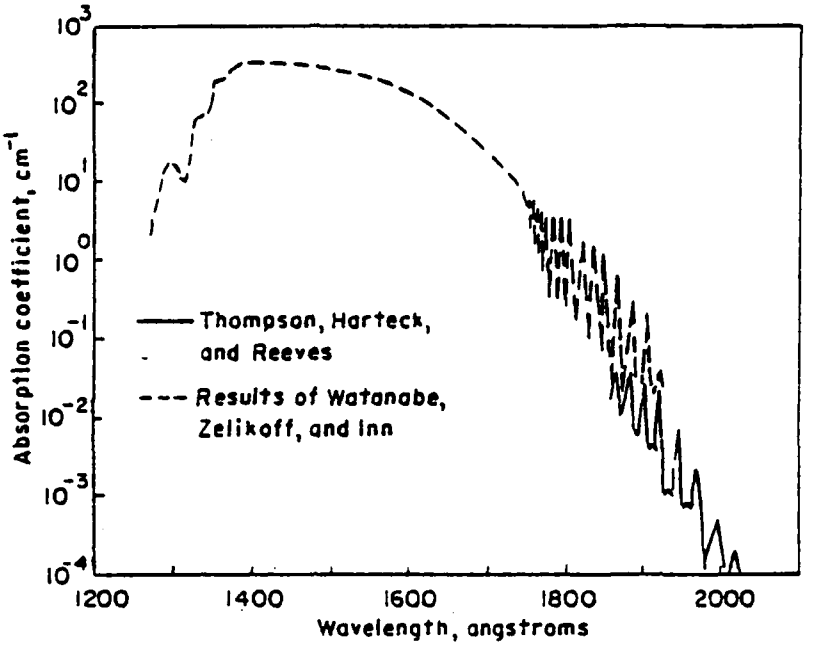


FIGURE 8 Absorption of O_2 as a function of wavelength.

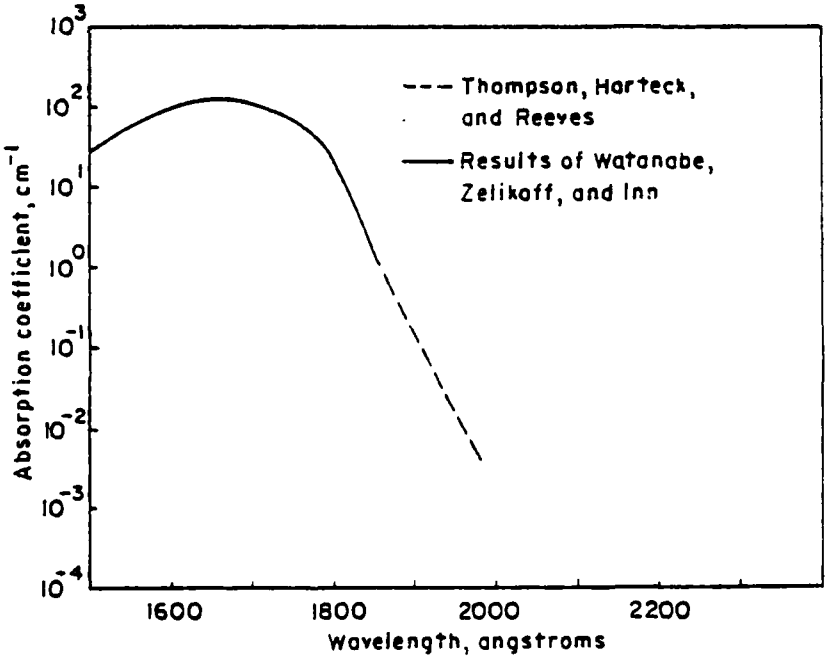


FIGURE 9 Absorption of H_2O as a function of wavelength.

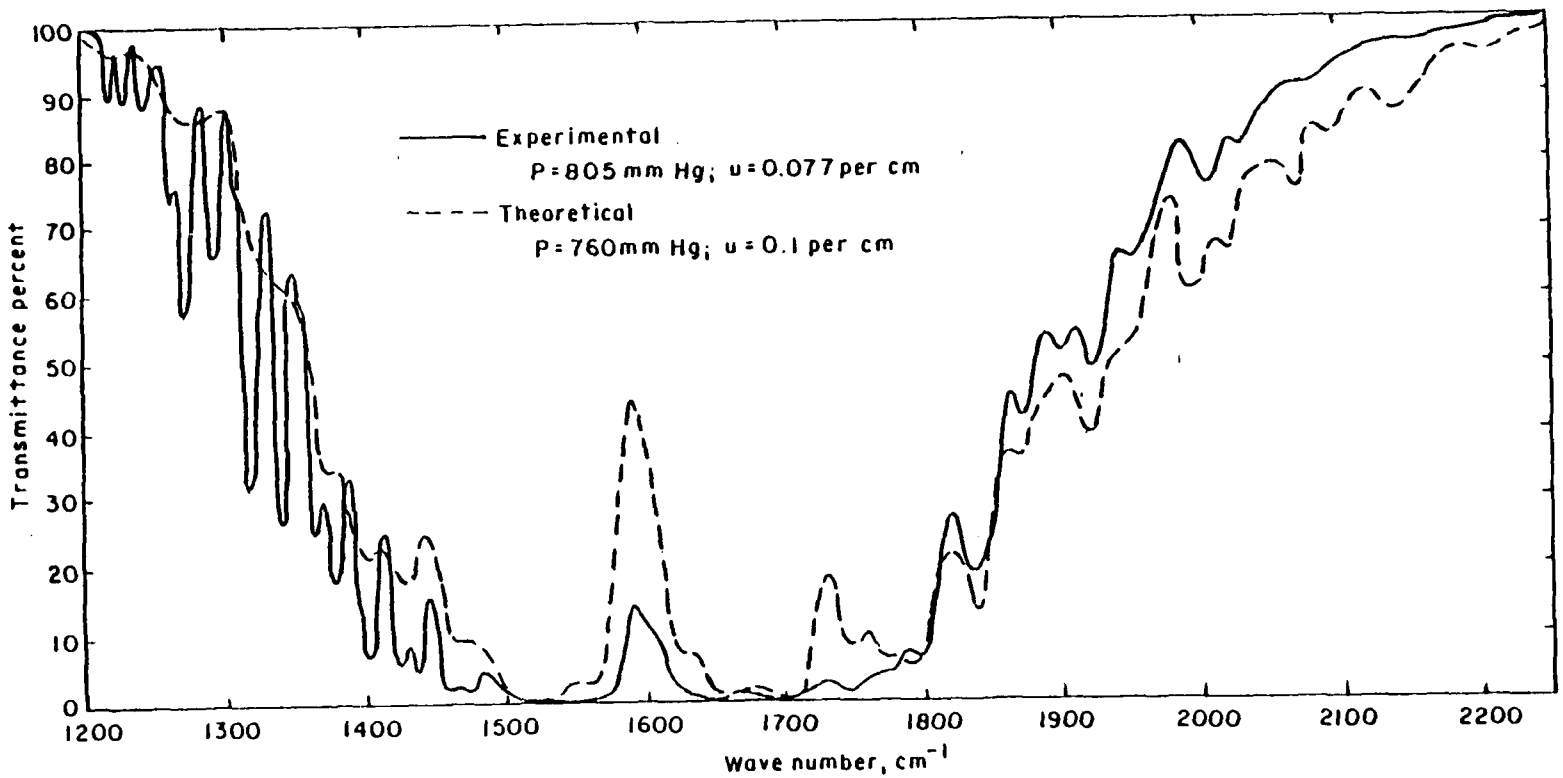


FIGURE 10 Comparison of the theoretical calculations of the transmittance of the 6.3-μ band with the experimental measurements of Burch et al. The theoretical values have been averaged over a 20 cm^{-1} interval.

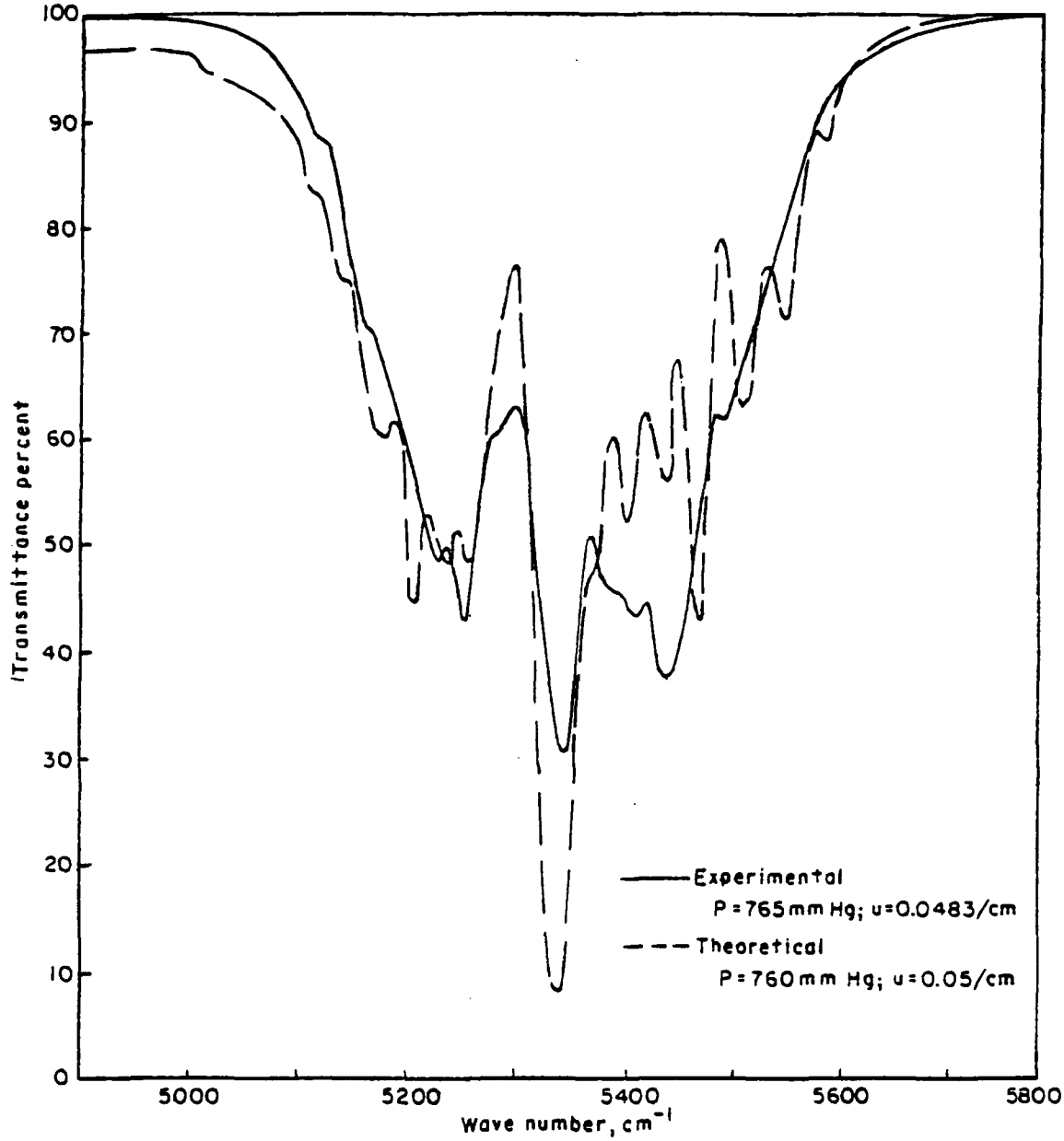


FIGURE 11 Comparison of the theoretical calculations of the transmittance of the 1.87-u band with the experimental measurements of Burch et al. The theoretical values have been averaged over a 20 cm^{-1} interval.

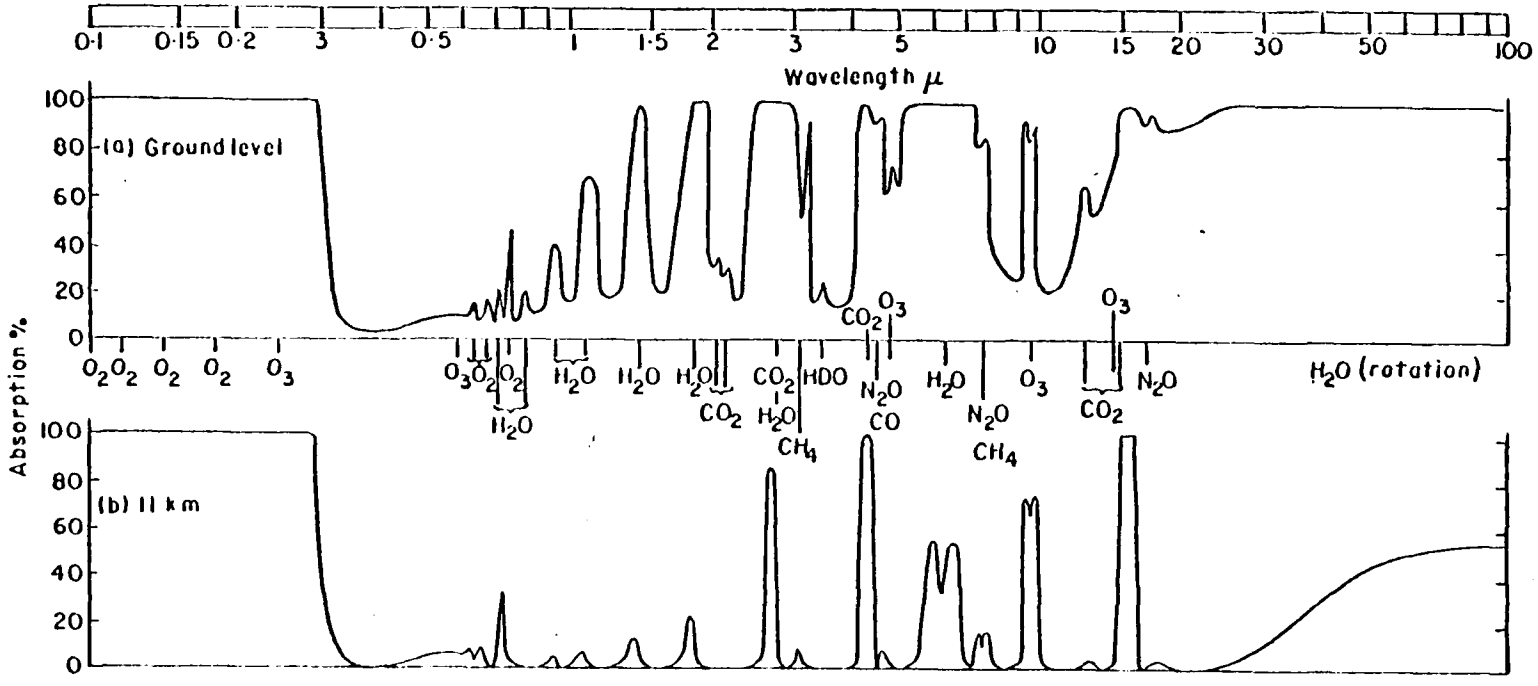


FIGURE 12 Atmospheric absorptions.
(a) Atmospheric gaseous absorption spectrum for a solar beam reaching ground leve. (b) The same for a beam reaching the temperature tropopause.

TABLE I

GAS PRESSURES USED FOR ABSORPTION MEASUREMENTS

(Thompson, Harteck, and Reeves)

Gas	Pressure, mm
CO_2	700
CO	660
O_2	720
H_2O	20
N_2O	326, 2050 - 4000 A 6, 1850 - 2050 A
NH_3	760, 2250 - 4000 A 100, 2250 - 4000 A 5, 1850 - 2150 A 0.1, 1850 - 2150 A
NO	6*
SO_2	5, 2000 - 4000 A 0.259, 1850 - 2200 A
CH_4	750

* NO was measured at low pressures to avoid interferences from dimerization.

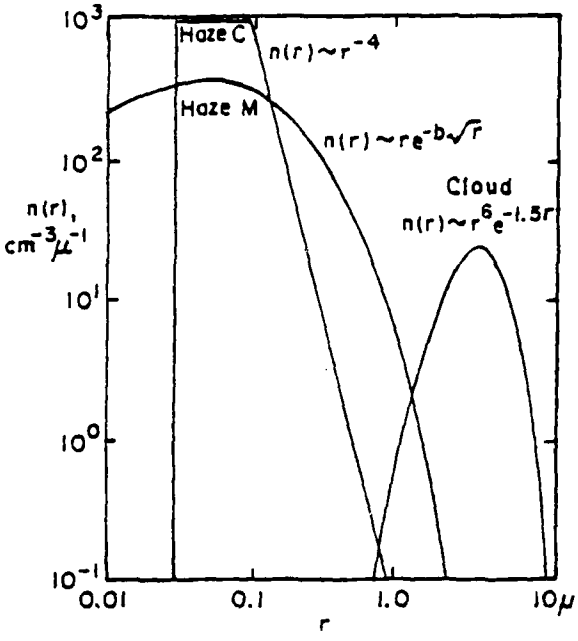


FIGURE 14 Three size distribution functions used in the integration of the Mie functions. Total concentration 100 cm^{-3}.

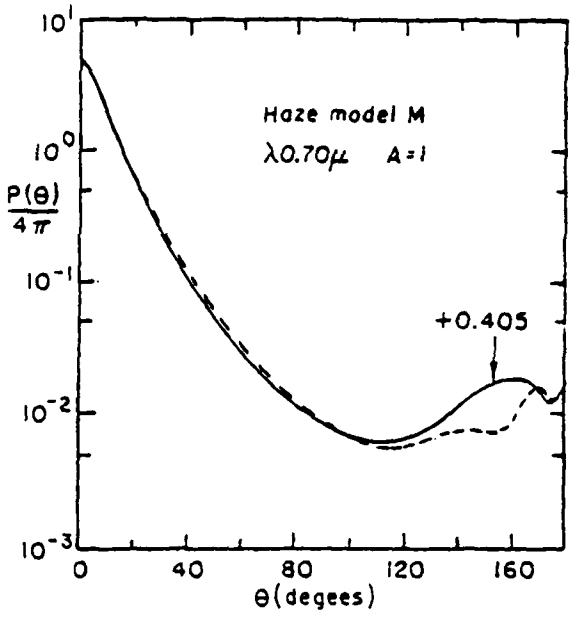


FIGURE 15 Intensity functions for haze particles at λ0.70μ and with real index 1.33 but a different distribution than Fig. . Computed values at θ: 0(2.5)20(10)130(2.5)180°.

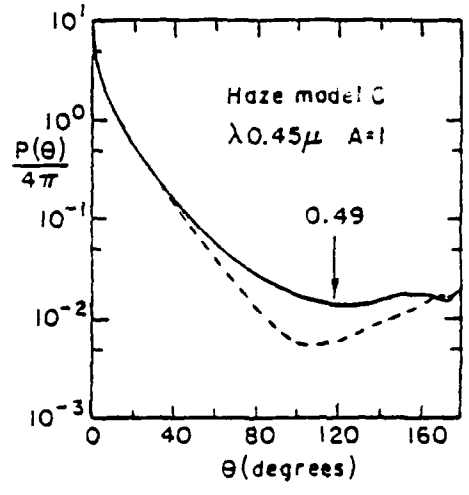


FIGURE 16 Integrated and normalized intensity functions $P_1/4\pi$ (solid line) and $P_2/4\pi$ (dashed line) for haze particles with complex index and infrared illumination. Computed values at θ: 0(1)5, 10(10)150(5)170(2)180°.

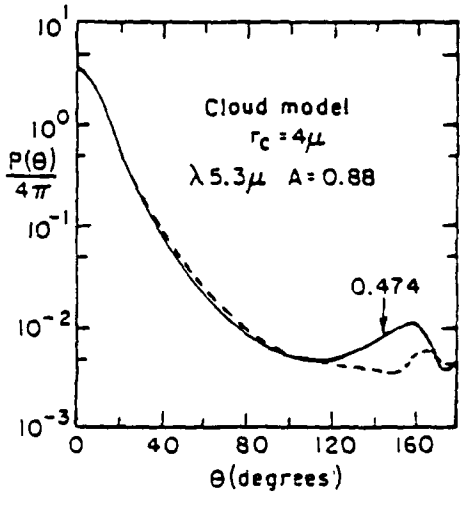


FIGURE 17 Intensity function for water cloud illuminated by 5.3μ radiation. Computed values at θ: 0(2.5)30(10)140(2.5)180°.

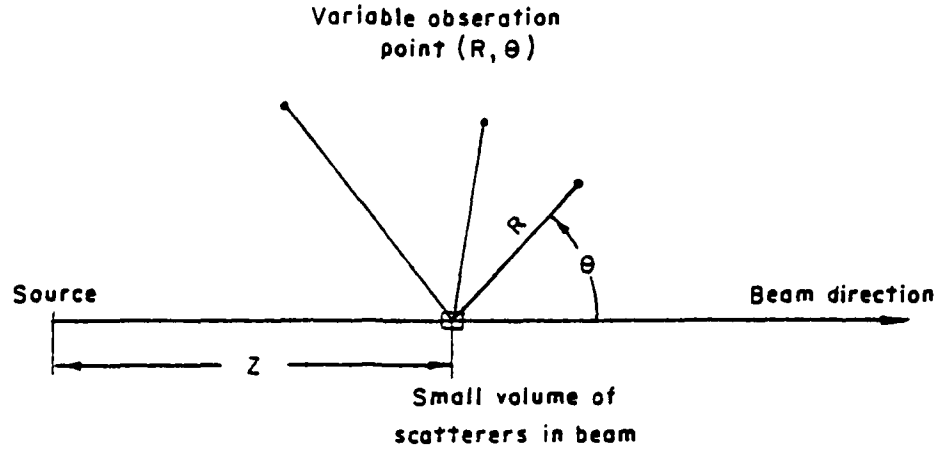


FIGURE 18 Scattering from small volume of scatterers, fixed scattering element and variable observation point.

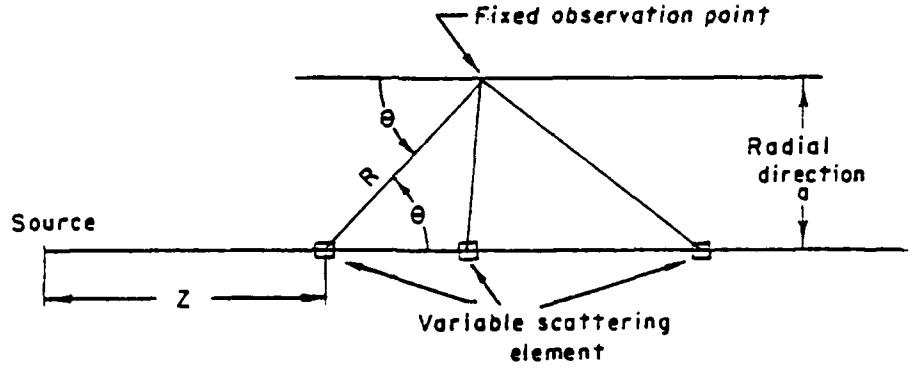


FIGURE 19 Scattering from a small volume of scatterers, fixed observation point, and variable scattering element.

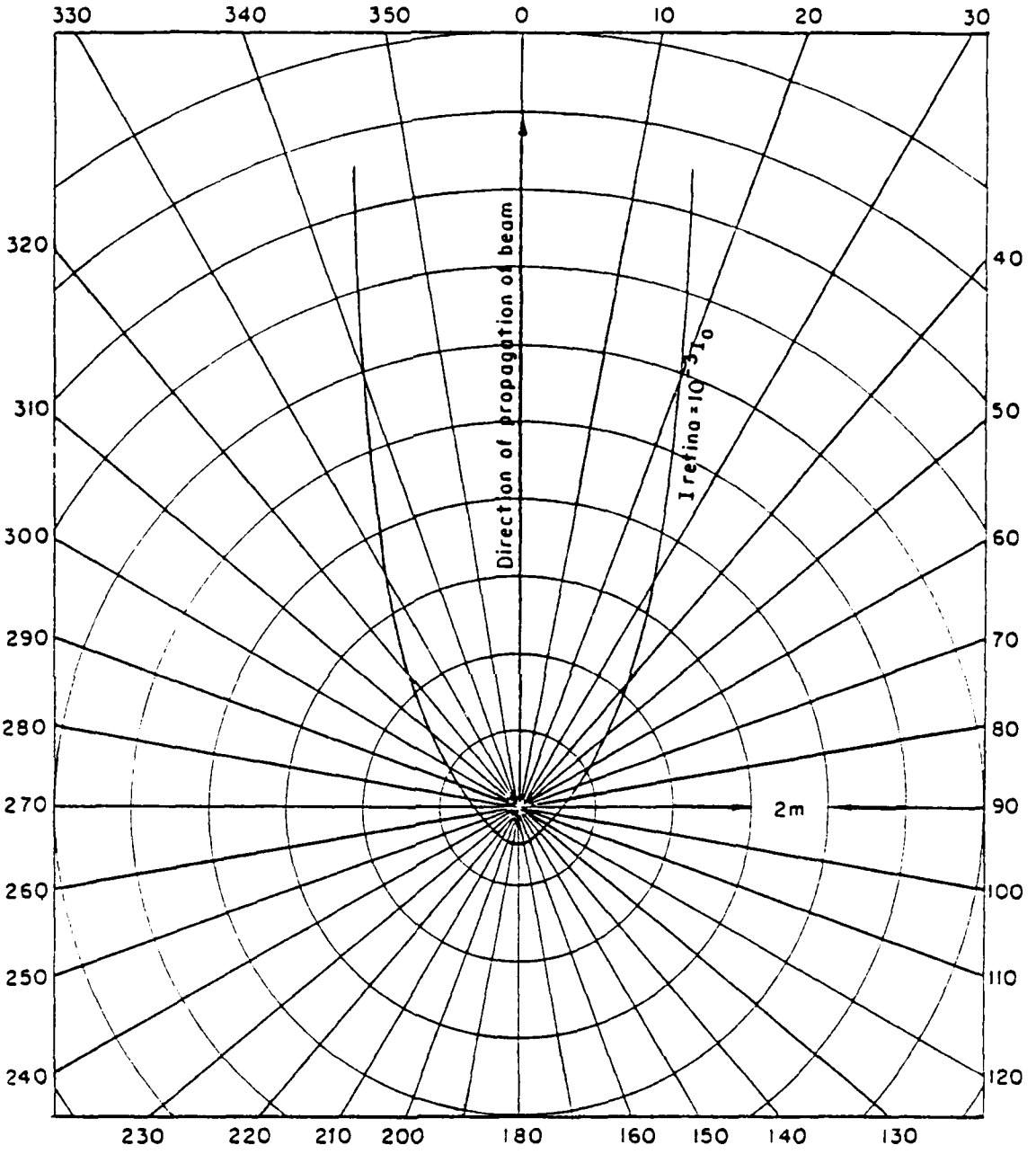


FIGURE 20 The angular distribution of light scattered from a small atmosphere volume within a beam of light.

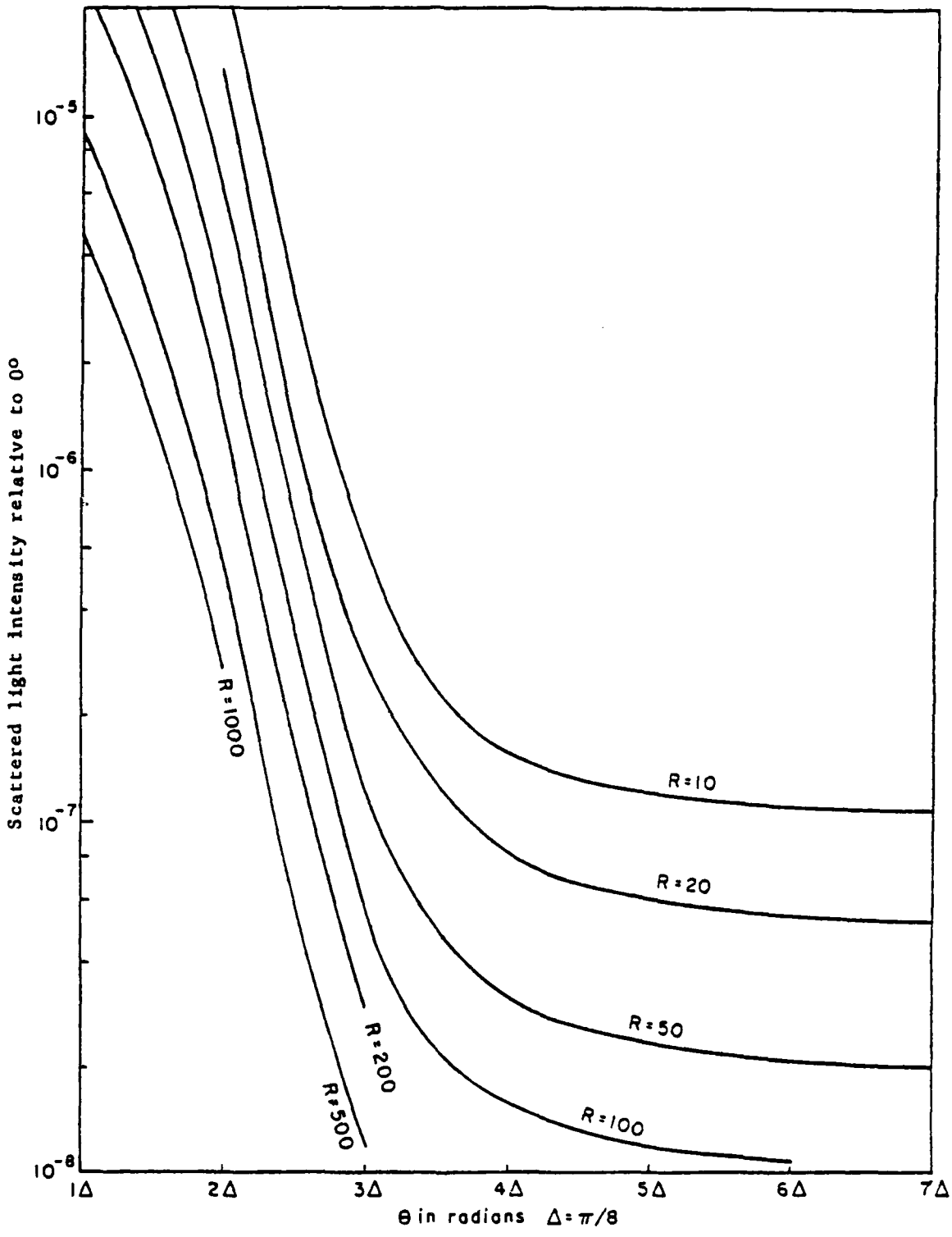


FIGURE 21 Total light scattered from a beam to an observation point (R,θ)

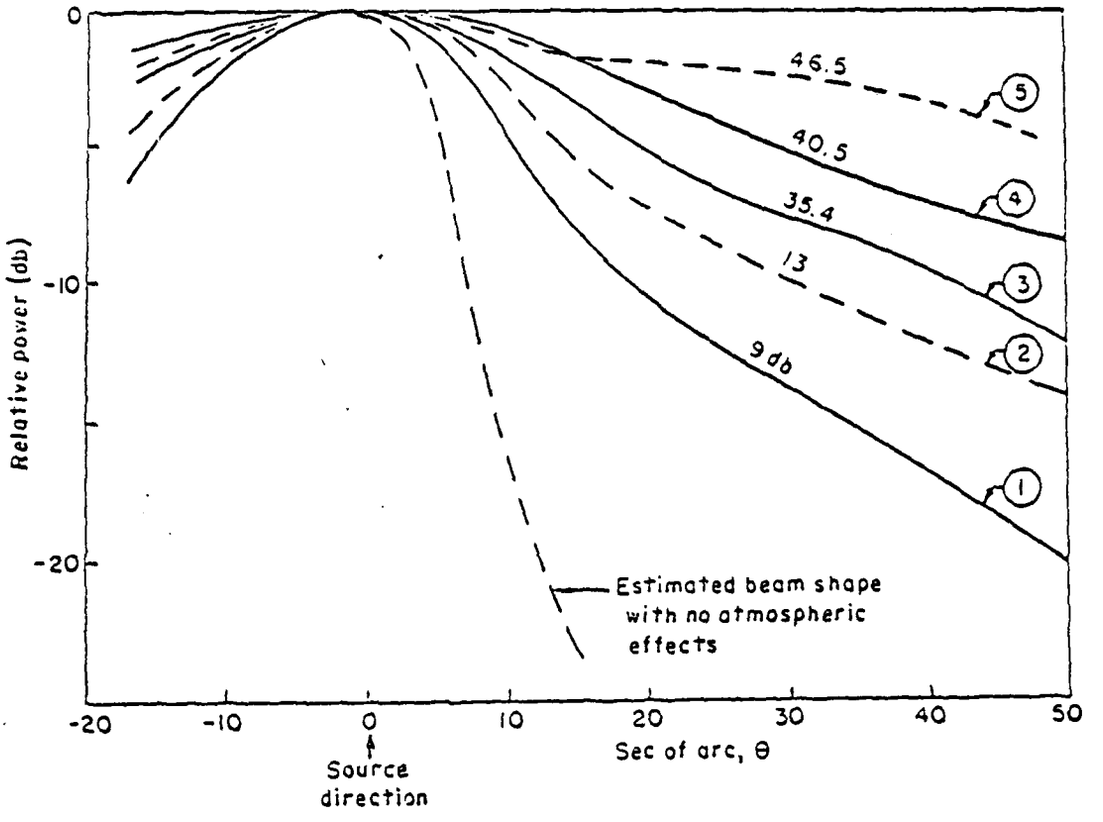


FIGURE 22 Beam broadening due to snow: , 0.63 ; path length, 2·6 km. Data of 2/10 and 2/11/64. Excess attenuations in curves 1-5 are 3·5, 5·0, 13·6, 15·6 and 17·9 db/km respectively, corresponding to increasingly heavy snowfalls. The attenuations are measured at dead reckoning, that is, with the receiving aperture on the maximum of the beam pattern. (ϑ=0)

(by D.C. Hogg)

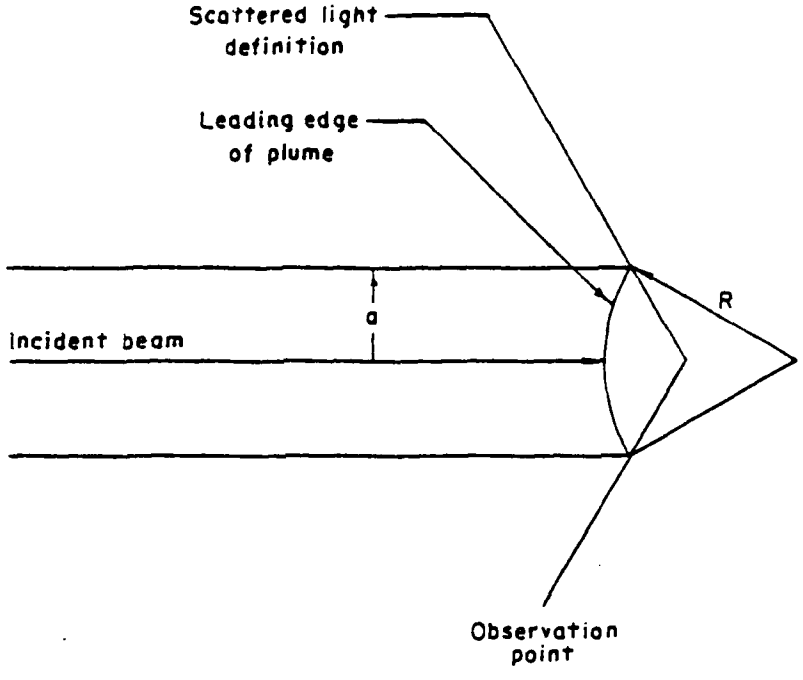


FIGURE 23 The scattering of a cylindrical beam by a totally reflecting plume.

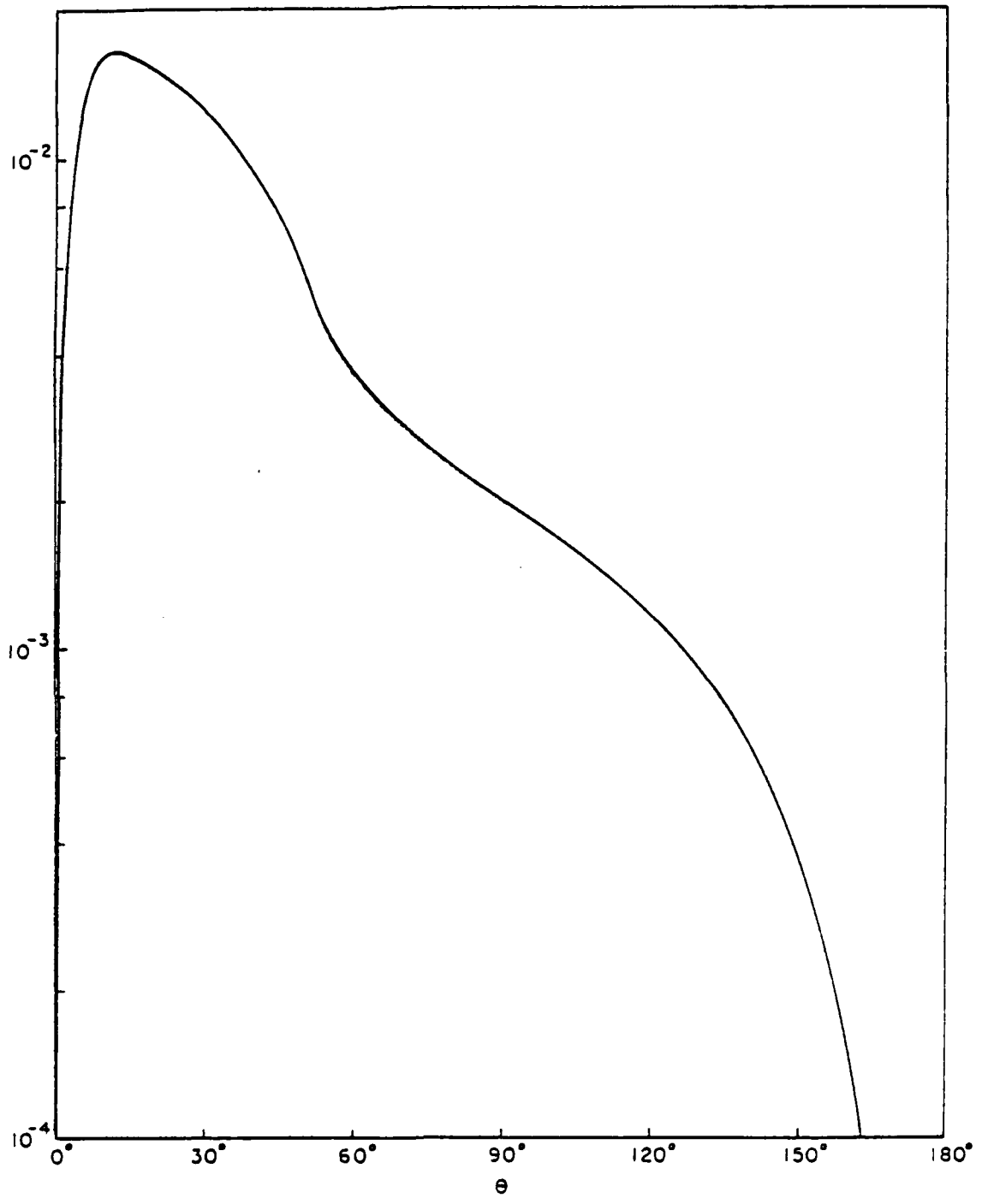


FIGURE 24 $F(\theta)\sin^2\theta \times 10^2$ vs θ

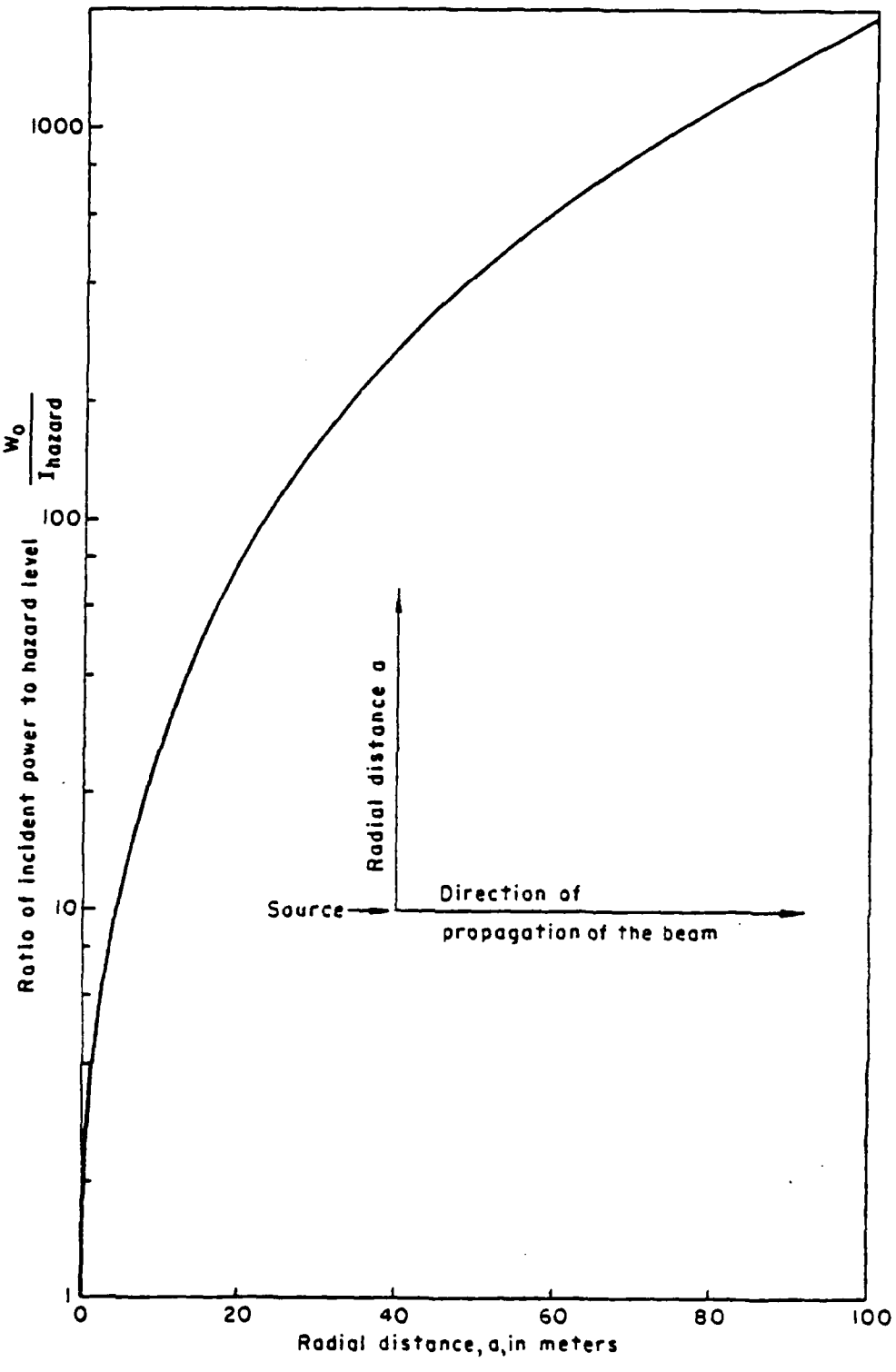


FIGURE 25 The ratio $\frac{W_o}{I_{hazard}}$ vs the safe radial distance for z = 0.

REFERENCES

1. Twersky, V., JOSA 52, 145-171 (1962)

1(a) Van der Hulst, Light Scattering by Small Particles, John Wiley-London (1957)

2. Van der Hulst, Light Scattering by Small Particles, John Wiley-London (1957)
3. Unpublished data
4. Thompson, B.A., et al., J. Geophys. Res. 68, 6431 (1963)
5. Green, A.E.S., Applied Optics 3, 203 (1964)
6. Wyatt, P.J., et al., Applied Optics 3, 229 (1964)
7. "The Infrared Absorption of Carbon Dioxide" - Report SSD-TDR-62-127, Vol. III, Space Systems Division, Air Force Systems Command, Los Angeles, Calif. (1963)
8. "The Infrared Absorption of Water" - Report SSD-TDR-62-127, Vol. II, Space Systems Division, Air Force Systems Command, Los Angeles, Calif. (1963)
9. Wyatt, P.J., et al., JOSA 52, 1209 (1964)
10. Burch, D.E., et al., "Infrared Absorption of Carbon Dioxide, Water Vapor, and Minor Atmospheric Constituents" - AF 191604, 2633, Ohio State University Inc., (December 1960)
11. Wyatt, P.J., et al., Applied Optics 3, 229 (1964)
12. Stull, V.R., et al., Applied Optics, 243 (1964)
13. Goody, R.M., Atmospheric Radiation, Oxford Clarendon Press (1964)
14. Green, A.E.S., Applied Optics 3, 203 (1964)
15. Gibson, G., Laughridge, F.I., Nichols, J.R., Drause, N.A., JOSA 52, 38 (1962)
16. Khostrick, et al., JOSA 52, 416 (1962)
17. Peck, E.A., Khanna, B.N., JOSA 52, 1010 (1962)
18. Van der Hulst, Light Scattering by Small Particles, John Wiley-London (1957)
19. Gumprecht, R.O., et al., JOSA 42, 226 (1952)
20. Hulbert, C.O., JOSA 31, 467 (1941)
21. Reege, E., Siedentopf, H., Optik 1, 15 (1946)
22. Bulrich, K., Moller, F., Optik 2, 301, (1947)
23. Deirmendjian, D., Applied Optics 3, 187 (1964)
24. Deirmendjian, D., Applied Optics 3, 187 (1964)
25. Khrgian, A., Mazin, I.P., Tr. Tsentr. Aerolog. Observ. 7, 56 (1952)
26. Khrgian, A., Mazin, I.P., Tr. Tsentr. Aerolog. Observ. 7, 36 (1956)
27. Mie, G., Ann Physik 25, 377 (1908)
28. Van der Hulst, Light Scattering by Small Particles, John Wiley-London (1957)
29. Reege, E., Siedentopf, H., Optik 1, 15 (1946)
30. See the section of this report titled "Laser Eye Protection"

31. Knestrick, G.L., et al., JOSA 52, 1010 (1962)
32. Eldridge, R.G., Johnson, J.C., JOSA 52, 7, 787
33. See section of this report titled "Laser Eye Protection"

34. Hogg, D.C., Nature 203, 396 (1964)
35. Hogg, D.C., Nature 203, 396 (1964)
36. Knestrick, et al., JOSA 52, 1010 (1962)
37. Edwards, B.N., Stean, R.R., Applied Optics 4, 331 (1965)
38. Bennet, H.E., Portens, J.O., JOSA 51, 123 (1961)
39. Morin, T.J., Peacock, G.R., "Microwave and Optical Plasma Diagnostics" AFCRL-64-903.
40. Southhall, P., Introduction to Physiological Optics, Oxford University Press - London (1937)
41. Southhall, P., Introduction to Physiological Optics,Oxford University Press - London (1937)
42. Reege, E., Siedentopf, H., Optik 1, 15 (1946)

Nonlinear Effects - Possible High Intensity UV Source

Franken and Ward (1) have postulated a schematic representation of optical polarization as an illustration of the mechanism for second harmonic generation. The optical polarization as a function of applied electric field is given by

$$P = XE\ (1+a_1\ E+a_2E^2\ \text{-----})$$

where X is the normal linear optical polarization (of order unity) E is the applied electric field and a_i (i=1,2,3,n) are nonlinear coefficients. If the applied field is $E = E_o \sin wt$ the quadradic term in the above expansion, p(2w), will be $p(2w) = xa_1E_o^2 \sin^2 wt = xa_2 (E_o^2/2)\ (1-\cos 2wt)$.

Since the second harmonic power is proportional to $p^2(2w)$(1a) it follows that

$$I\ (2w) \propto p^2(2w) \propto E_o^4 \propto I^2\ (w)$$

or the second harmonic power density is proportional to the square of the incident power density. Since a_1 is a very small, appreciable second harmonic generation will occur only at high incident power densities. Theories of second harmonic generation (2,3) indicate that only structures lacking inversion symmetries will result in second harmonic generation. However, effects of molecular interaction on local symmetry has been observed (4) to result in frequency doubling from molecules which would not otherwise support the mechanism.

Second harmonic generation in liquids has been reported by Terhune et al (5). A 1 megawatt peak power, 80 nsec. Q-switched ruby laser output

was focused in H_2O, $C Cl_4$ and CH_3CN which had been filtered through 5.μ millipore filters. It was reported that about 10^{-13} the incident energy emerged as scattered radiation near twice the laser frequency. From the diagram of the arrangement of equipment used in this experiment it is concluded that the second harmonic signal measured was the power radiated to $90^{\circ} \pm 20^{\circ}$ from the direction of propagation of the incident beam. No information was given concerning second harmonic generation at other angles, and it was not indicated how the measured value and the ratio of second harmonic energy to incident energy were related. Since the total second harmonic generation appears to be low, only minimal hazard is anticipated from second harmonic generation in liquids unless more efficient conversion efficiencies are found.

Conversion efficiencies in crystals can be much greater than those in liquids, provided the crystals lack an inversion symmetry and possess equal refractive indicies at fundamental and second harmonic frequencies (6,7). The latter condition is met by proper orientation of crystals as described by Giordmaine (8) and Maker et al (9).

P.D. Maker et al (10) have observed that by focusing incident radiation a gain of $3X10^3$ in second harmonic intensity may be realized over the unfocused value. It is estimated that this gain was composed of a factor of 100 due to increased power density and a factor of 30 due to mixing (beating) of divergent rays. Consequently one may expect increased conversion efficiency under focused conditions, greater than that expected from the increased energy density. Upon application of these principles, conversion efficiencies as high as 20% have been achieved by focusing a Q-switched ruby laser within a properly oriented block of KH_2PO_4(KDP) (11).

Efficient second harmonic generation in other crystals such as Li Nb O_3 (12) and KD_2PO_4 (13) has also been observed.

Johnson (14) has produced ultraviolet radiation (2650 Å) from infrared (1.06μ) by quadruplying the frequency of a Q-switched 10 megawatt Neodymium-in-glass laser using two KDP crystals in sucession. The conversion efficiencies were 2% from the IR (1.06μ) to visible (5300 Å) and 1% from the visible to UV (2650 Å) resulting in a combined efficiency of 0.02%. Johnson points out that the experiment was performed with the incident laser beam unfocused to prevent damage to the KDP crystal. He estimated that had the beam been focused, conversion efficiency at the first crystal would have been approximately 20% and the resulting increase in incident radiation at the second crystal would have increased its efficiency also. The ultraviolet power level at 2650 Å, would consequently have been raised from the observed level, 2.4 Kw, to approximately 100 Kw (15).

Efficient second harmonic generation in optically active amino acids has been reported by Rieckhoff and Peticolas (16). The ultraviolet signals were established as a second harmonic rather than some other luminescence by accurate determination of spectral content. A very narrow line at the second harmonic frequency was interpreted as sufficient evidence of second harmonic generation rather than florescence. A 10 megawatt Q-switched ruby laser (6943 Å) was focused 7cm behind the sample to be tested with a 15 cm focal length lens resulting in roughly 40 megawatts/cm^2 at the sample. The samples were in the form of a layer of powder 1mm thick, enclosed between a microscope glass slide and a cover glass. The authors indicated that the conversion efficiency of the amino acids tested were as much as three and four times that of KDP. This statement is highly questionable since the conversion efficiency of KDP can be as high as 20%. The authors indicated

that particular acids may be capable of supporting the mechanism when in the form commonly found in cells. No experimental work of this nature was reported however.

Another nonlinear mechanism, Raman scattering, results in secondary emission at frequencies other than harmonics of the incident radiation. Following interaction of a quantum of light energy, hv_o, with an atom of molecule of energy E_o, the atom or molecule is excited to state E_2. If it returns to its original state with emission of a photon the energy of that photon is hv_o and Rayleigh scattering is produced. If it returns to a state of other than the original, E_1 then emitted photon will have energy $hv_1 = E_2 - E_1$. When $E_1 > E_o$, $hv_1 < hv_o$ and the energy and frequency of the emitted photon are less than those of the incident photon. If $E_1 < E_o$, $hv_1 > hv_o$, and the emitted photon is of higher frequency and energy than the incident photon. The former case is referred to as Raman Stokes radiation and the latter, Raman anti-Stokes Radiation.

Most Raman scattering results from spontaneous emission in which case the scattered radiation will be incoherent and diffuse. However, if the Raman radiation is present in sufficient intensity or if the Raman and exciting radiation are incident on the medium simultaneously, stimulated Raman scattering will occur. A detailed review of both experimental and theoretical work concerned with Raman scattering has been compiled by Stoicheff (17) and detailed theory of stimulated Raman scatter is discussed by Garmire et al (18) and Javan (19).

Some of the interesting properties of stimulated Stokes-Raman scattering have been reviewed by Eckhardt et al (20). They report an apparent threshold for stimulated Raman radiation production. The resulting radiation is coherent, collimated in the direction of the incident beam and is

contained in a series of narrow spectral lines. The intensity of the stimulated radiation was reported to be as high as 10% the incident radiation. Stimulated anti-Stokes-Raman radiation has been reported by Terhune (21) and Stoicheff (22). The observed stimulated anti-Stokes radiation was of intensity comparable to that of the incident beam and was confined to cones 10 min (0.157^{o}) wide within a few degrees of the forward direction. Several orders of anti-Stokes radiation were observed, all of narrow line width. As with stimulated Stokes-Raman scattering there was an apparent threshold for stimulated anti-Stokes radiation.

Hazards may arise from nonlinear effects in both experimental targets and biological material. Rieckhoff and Peticolas (23) have noted that ultraviolet radiation generated due to nonlinear effects at specific sites in biological substances may be absorbed by surrounding constituents. Consequently it is concluded that media which are normally optically inactive at the frequency of incident radiation may be effected when nonlinear effects are significant.

Production of ultraviolet radiation due to nonlinear and Raman effects in experimental targets could possibly present a hazard to exposed surfaces of observers. This is due to the fact that the absorption spectra for biological media α abs (λ), including the superficial layer of the eye, tends to increase in the near ultraviolet.

By dissection of a rabbit eye and reassembling the various components in quartz envelopes in a manner such as to duplicate their original geometry, Kinsey (24) attempted to measure the absorption characteristics of ocular

constituents in the ultraviolet region. He concluded from his measurements that almost all of the light (in the UV) incident on a whole rabbit eye which is not reflected will be absorbed before reaching the retina. No information was reported concerning the fraction of incident light that was reflected. Absorption measurements made with this technique may not necessarily yeild information concerning the fraction of incident radiation absorbed in the different parts of the reconstructed globe. Kinsey observed that abosrption in the aqueous and vitreous humor, and in the cornea, peaks at 3000 Å while the corneal epithelium absorbs most strongly at 2900 Å.

Veroeff and Bell (25) have noted that due to high ultraviolet absorption of the anterior part of the eye adverse effects will depend on the total energy density at the surface from all angles rather than the amount of incident from specific angles as in the case in the visible region. Consequently, in contrast to visible radiation, the effects of a distributed source of radiation are much the same as those of a point source.

The threshold for damage to the corneal epithelium was found by Cogan and Kinsey (26) to be approximately 0.01 joule/cm^2 at peak sensitivity (2800 Å) while Verhoeff and Bell (27) found that the threshold for nonmonochromatic ultraviolet light was roughly ten times that required at peak sensitivity. Unfortunately no reference was made to time dependence in either study (or spectral distribution in the study of Verhoeff and Bell). Although damage at these levels is to a great extent reversible, one may consider 0.01 joules/cm^2 a hazard level since at least temporary visual impairment will be associated with the resulting disturbances.

Ocular shielding which will allow safe observation of experiments becomes extremely difficult when inelastic (frequency shifted) scattering

is significant, This is due to the fact that resulting radiation will be contained at several wavelengths in addition to that of the incident radiation. The optical shield would consequently be required to attenuate light only at a number of discrete frequencies.

REFERENCES

1a. Corson, D., Lorrain, P., Introduction to Electromagnetic Fields and Waves, W.H. Freeman and Co., San Francisco (1962)

1. Franken, P.A., Ward, J.F., Review of Modern Physics 35, 23 (1963).

2. Yin-Yan, L., Acta Physica Sinca,20, 164 (1964).

3. Kielich, S., Bulletin De L'Academie Polonaise des Sciences XII 53 (1964).

4. Terhune, R.W., Maker, P.D., Savage, C.M., Phys. Rev. Letters 14, 681 (1965).

5. Kielich, S., Bulletin De L'Academie Polonaise des Sciences XII 53 (1964).

6. Terhune, R.W., International Science and Tech., p. 38 (August 1964).

7. Maker, P.D., Terhune, R.W., Nisenoff, M., Savage, C.M., Phys. Rev. Letters 8, 21 (1962).

8. Giordmaine, J.A., Phys. Rev. Letters 8, 19 (1962).

9. Maker, P.D., Terhune, R.W., Nisenoff, M., Savage, C.M., Phys. Rev. Letters 8, 21 (1962).

10. Maker, P.D., Terhune, R.W., Nisenoff, M., Savage, C.M., Phys. Rev. Letters 8, 21 (1962).

11. Giordmaine, J.A., Scientific American, 210, 38 (1964).

12. Boyd, G.C., Milles, R.C., Nasark, Bond, W.L., Savage, Applied Phys. Letters 5, 234 (1964).

13. Van der Ziel, J.P., Bloembegen, N., Phys. Rev., 135 (1964).

14. Johnson, F.M., Nature, 204, 985 (1964).

15. Johnson, F.M., Nature, 204, 985 (1964).

16. Riechoff, K.E., Peticolas, W.L., Science, 147, 610 (1964)

17. Stoieheff, B.P., Stimulated Raman Scattering, Division of Pure Physics, National Research Council, Ottowa.

18. Barmire, E., Pandarese, F., Townes, C.H., Phys. Rev. Letters 11, 160 (1963).

19. Javan, A., Stimulated Raman Scattering, Division of Pure Physics, National Research Council, Ottowa, p. 284.

20. Eckhardt, G., et al, Phys. Rev. Letters 9, 455 (1962).

21. Terhune, R.W., Bull. Amer. Phys. Soc. 8, 359 (1963).

22. Stoicheff, B.P., Stimulated Raman Scattering, Division of Pure Physics, National Research Council, Ottowa.

23. Riechoff, K.E., Peticolas, W.L., Science 147, 610 (1964).

24. Kinsey, V.E., Arch. Industrial Health 11, 305 (1955).

25. Verhoeff, F.H., Bell, L., Proc. Amer. Acad. Arts and Sciences, Boston (1916).

26. Kinsey, L.E., Cogan, D.G., Drinken, P., J.A.M.A. 123, (October 16, 1943)

27. Boyd, G.D., Milles, R.C., Nasark, Bond, Savage, Applied Phys. Letters 5, 234 (1964).

Gas Lasers

The first sustained, continuous laser output was produced using a gas mixture, the helium-neon system, which operated in the infrared with an output power on the order of one milliwatt (1). In 1962, a review paper by Bennett (2) listed some fifty-three gas laser wavelengths in pure gases and mixtures. Laser transitions were identified in neon, cesium, oxygen, helium, argon, krypton, and xenon. Today, over 500 transitions have been observed in various gases and vapors and it seems likely that this number will increase significantly over the next three years.

The high coherence properties, directionality, stability, and range of wavelengths of gas and vapor lasers suggest many applications. In addition to the improved optical instruments that can be fashioned by and with them, gas lasers have many potential uses in communications, range finding, plasma diagnostics and optics. In fact, the gas laser comes closest to exhibiting all the properties of an ideal laser. Although peak powers do not approach those attained with the ruby or neodymium systems, the gas laser is widely used in research and teaching.

The present state of the art of gas lasers is summed up very well in a recent paper by Bennett (3). At present more than 500 lines are known to exhibit laser action either continuously or under pulsed exitation. Laser wavelengths range from the ultraviolet (neon 2678.8A) to the infrared (HCN 337 microns). Power outputs may be: continuous, from a fraction of a milliwatt to almost 20 watts (ionized argon); pulsed, 20-40 nanoseconds, up to 100 watts (nitrogen); quasi-CW, several watts for several millisecond pulses in argon. Lasing action has been observed in the following gases and vapors, over the wavelength ranges indicated (3).

1. Argon:	86 lines (strong lines from 3511-5145A)	2753 A-26.95u
2. Bromine:	4 lines in region of 8446A	
3. Carbon:	9 lines	4647 A-5.5956u
4. Cesium:	2 lines	3.204u and 7.1821u
5. Chlorine:	11 lines	4781 A-2.20603u
6. Helium:	2 lines	1.9543u and 2.0603u
7. Iodine:	8 lines	5407.4 A-3.431u
8. Krypton:	59 lines	3050 A-7.0565u
9. Mercury:	25 lines	4797 A-1.813u
10. Neon:	155 lines (line at 3.39u has gain 40dB/meter)	2678.6A-132.8u
11. Nitrogen:	9 lines	3478.7A-1.4547u
12. Oxygen:	13 lines	2984.6 A-8446.37A
13. Sulfur:	2 lines	1.0455u and 1.0636u
14. Xenon:	64 lines (line at 3.507u has highest gain 60 dB/meter)	2983.8A - 18.5u
15. CO (Carbon Monoxide)	three visible bands	5590.6 A-6613.5u
16. CO_2 (Carbon dioxide)	two bands	9u and 10u
17. D_2O (Deuterium Oxide)	16 unidentified transitions	from 33.9u-107.7u
18. N_2	4 bands	8683.5A-1.2347u
19. Ammonia (NH_2, NH_3, NH_4)	7 wavelengths	21.47u-31.95u
20. H_2O (water vapor)	(32 unidentified transitions)	
21. N_2O (Nitrous Oxide)	one band	region of 10.8u
22. HCN (Hydrogen Cyanide)		region of 337 microns

Hazards

The dispersion or beam spread of the laser beam from gas lasers is extremely small, characteristically in the range of ten milliradians or less (see Fig. 1). Thus many gas lasers can be hazardous to the eye. Retinal damage thresholds for visible gas laser radiation have not been firmly established at any wavelength. However, it seems reasonable to assume approximately 1 joule/cm^2 as a hazard threshold at a diffraction limited retinal spot of the order of 100u^2, based on the comprehensive studies of Ham and Geeraets (8,9). In this approximation a milliwatt laser will produce a radiant power density at the retina of 10^3 watts/cm^2 which will deliver a total energy density of 1 joule/cm^2 in 10^{-3} seconds, or the apparent threshold for damage. This also shows that the low power lasers of 100 microwatt power output may be hazardous in that a retinal lesion could possibly be caused by a 10 milli-second exposure, a time short compared to the blink reaction time which is of the order of 100 milli-seconds (10). The exact thresholds for optical retinal damage with gas lasers and other lasers are far from established. Recent studies indicate that retinal irreversible injury thresholds for pulsed irradiation may be as low as 0.14 joules/cm^2, non-Q-switched ruby at 6943 A (11), and as low as 0.07 joules/cm^2, Q-switched. Further studies in this area is needed since the number of applications of low power gas lasers is increasing. Both irreversible and reversible eye damage thresholds for infrared and ultraviolet lasers also have not been established, although liminal dosage for the onset of conjunctivitis due to non-laser ultraviolet wavelengths has been studied as is pointed out in the section on Flash Lamps. The hazards here may be to regions of the eye other than the retina and conjunctiva depending on the absorption characteristics of the various

regions.

There is some specific information available concerning skin erythema and its production by ultraviolet radiation of different wavelengths (12,13). This in itself is a distinct hazard, usually reversible, but can be painful. The possible carcinogenic effects of such radiation consequently cannot be overlooked as a long term hazard. The threshold of ultraviolet erythema has been established at various wavelengths (12,13), and at the most sensitive wavelength (Table I), 2967 A, is 430 microwatts/cm^2 for 2 minutes exposure. Extrapolating under the assumption of reciprocity (cumulative exposure-energy-density), this threshold may be stated as 5.16×10^{-2} joules/cm^2. Thus even milliwatt continuous outputs of any of the lasers listed as producing wavelengths shorther than 3000 A can present serious erythemal, and possible carcinogenic, hazards. For example, at the 3002 A line in Argon II, a one milliwatt laser output, focused to a 0.1 mm spot would deliver 10 watts/cm^2 to the region. Using an erythemal effectiveness of 50% (Table II), a one-tenth second exposure should produce a perceptible erythemal response on normal untanned skin.

Perhaps the next most important hazard of gas lasers is the fact that users are not properly warned concerning the potential hazard of viewing even a ten times attenuated one milliwatt He-Ne beam. The relatively low power output of small gas lasers does not suggest the potential hazard than can exist.

Another hazard associated with gas lasers is the reflected beam from the discharge tube windows. Most gas lasers have discharge tubes terminated at the ends by Brewster angle windows. The windows are set at a specified angle, the Brewster angle, to the discharge tube axis (about 35°), in order that the

laser amplifies only a selected polarization (See section on Polarization). In open structures, where the Brewster angle windows are not shielded, a certain fraction of the beam is reflected at both windows out of the optical cavity at an angle of approximately 70° to the beam axis. The presence of these reflected beams, nearly orthogonal to the laser axis, of lower power than the main laser beam can increase the potential hazards in the laboratory. However, proper shielding can eliminate this hazard.

Other hazards to be considered with respect to personnel are: back-scatter radiation from targets, possible carcinogenic effects of ultraviolet radiation from both direct short wavelength laser irradiation and radiation emitted from unshielded quartz discharge tubes. This latter radiation hazard, unevaluated as yet, may be particularly significant in the high repetition rate pulsed gas lasers and ionized gas lasers where high density plasmas are present. However, plasmas are also present in most other gas laser discharge tubes. The He-Ne gas laser in particular ordinarily uses a quartz or vycor tube discharge tube and in most commercial units is, at best, only partially shielded. Since quartz and vycor transmit ultraviolet with wavelengths as short as 2000 A, ultraviolet radiation is present, but is, as yet, unevaluated with respect to intensity and spectral distribution. The spectral distribution of ultraviolet radiation is important in skin erythema considerations and may have similar importance in ophthalmological considerations. Table I shows the relative effectiveness for the production of skin erythema with ultraviolet radiation (12).

Ultraviolet gas lasers should be shielded since prolonged exposure to such radiation from continuously operating discharge tubes may be hazardous.

Gas Laser Potential for Next Three Years

The estimates made by Bennett (2) in 1962 when compared to his recent

review of the state of the art of gas lasers (3) show a remarkable change in the field. His estimates of power to be achieved have been exceeded by almost an order of magnitude. The additional laser transitions found in the last three years is a full order of magnitude increase from 1962. In short, the whole area of gas lasers has expanded very rapidly. Two significant contributions to the expansion are the non-steady state inversions attained in fast pulsed discharges, and the increased powers attainable in ionized gas lasers (4,5,6).

Most gas lasers are four level systems. That is, a population inversion is maintained between two levels, #2 and #1. The upper level, #2, undergoes stimulated emission to the lower state #1. State #1 is depopulated by spontaneous emission, not to the ground state, but to an intermediate, long lived, metastable state #3. Before the atom can again contribute to laser action it must return to the ground state #4, where it can be repumped to state #2. The fact that the intermediate state #3 is metastable hinders the decay of this state. Processes must come into play which can cause an atom in the metastable state to return to state #4. In these circumstances the population inversion density versus pumping rate can saturate or even decrease. This so called metastable "bottle-neck" has prevented most lasers from achieving higher power outputs or more efficient operating conditions. The ionized gas laser seems to be the first step in overcoming this metastable drawback. Although efficiency is low, 0.010%, no distinct saturation limit on pumping rate versus power output has yet been observed. It seems readily apparent that ion lasers will increase their power outputs by one or two orders of magnitude in the next three years, achieving power levels of 100 watts to 1 kilowatt continuous. However, efficiency may possibly be increased by a

factor of 10 or 100 which would still be a low efficiency, relative to semiconductor lasers.

In addition to the ion lasers, a new class of lasers has been proposed (3,7) called collision lasers, wherein the metastable problem can be overcome by playing various atomic collision processes against one another. According to Bennett (3), power outputs of a few watts/cm^2 per unit lasing volume at 50% efficiency is not an unreasonable figure to consider as possible. This could possibly permit power levels of the order of 10 to 100 watts continuous to be achieved with this system. Gould (7) also discusses potential designs of gas lasers with efficiencies of 20% or better. He also suggests the possibility of multijoule pulsed gas lasers operating in the infrared. It is indeed very likely that high power output gas lasers operating in the infrared will become available in the next few years. This potential is further enhanced by the recent operation of a 16 watt, CO_2N laser at 40% efficiency in the region of 10 microns by Patel (14).

TABLE I

The relative spectral erythemic of the untanned human skin, of average pigmentation, to equal amounts of radiant energy. (From Coblentz)

Wavelength (Angstroms)	Response
2400	0.56
2500	0.57
2600	0.42
2700	0.14
2804	0.06
2900	0.31
2950	0.98
2967	1.00
3000	0.83
3100	0.11
3200	0.005
3300	0.000

1. Javan A., et. al., Phys. Rev. Letters, 6 (3): 106, 1961

2. Bennett, W.R., Jr., Applied Optics - Optical Maser Suppl., p.24, 1962

3. Bennett, W.R., Jr., Applied Optics, Chemical Laser Suppl., p.3, 1965

4. Gordon, E.I., et. al., Appl. Physics Lett., 4 (10): 178, 1964

5. Bennett, W.R., Jr., et. al., Appl. Phys. Lett., 4 (10): 180, 1964

6. Bridges, W.B., Appl. Phys. Lett., 4 (7): 128, 1964

7. Gould, Appl. Optics - Chemical Laser Supply, p. 59, 1965

8. Ham, W.T., Geeraets, W.J., et. al., "Electronically Pulsed Light Sources for the Production of Retinal Burns", Amer. J. of Med. Electronics, Vol. 2 #4, 308, 1963

9. Geeraets, W.J., Ham, W.T., et. al., "Laser versus Light Coagulator: A funduscopic and histologic study of chorioretinal injury as a function of exposure time", Fed. Proc., Vol. 24 #1, part III, S-48, 1965

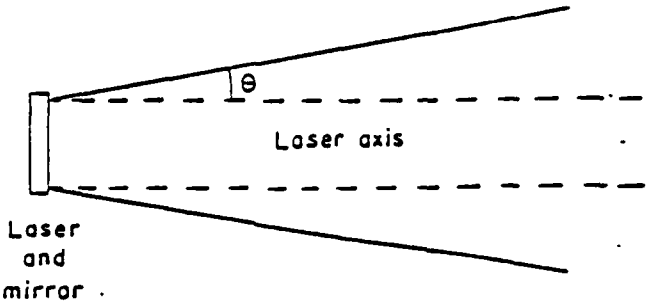
10. Christner, C.R., et al, "State-of-the-Art Study on Visual Impairment by High-Intensity Flash of Visible, Infrared, or Ultraviolet Light", Report No. BAT-171-9, Batelle Memorial Institute, Jan, 1965

11. Jones, A.E., McCartney, A.J., "Pulsed Ruby Laser Effects on Primate Ocular Structures", Biomedical Laser Conference, Boston, June, 1965

12. Luckiesh, M., Taylor, A.H., "Erythemal and Tanning Effectiveness of Ultraviolet Energy", G. E. Review, p. 274, June 1939

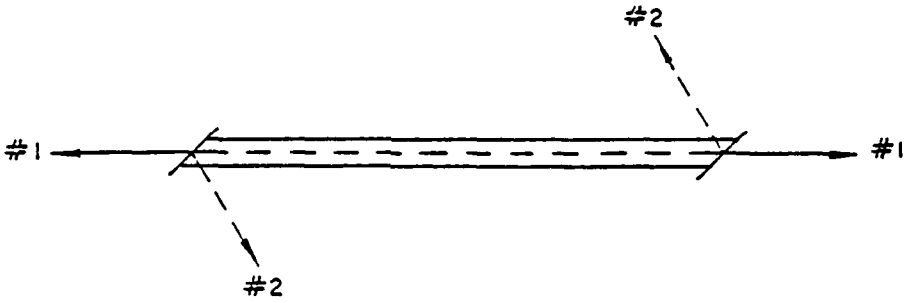
13. Coblentz, W.W., Stair, R., Hogue, J.M., "The Spectral Erythemic Reaction of the Untanned Human Skin to Ultraviolet Radiation", U.S. Bur. of Standards Journal of Research, Vol. 8, p. 541, 1932

14. The Laser Letter, Vol. 2, No. 11, p. 2, June 30, 1965

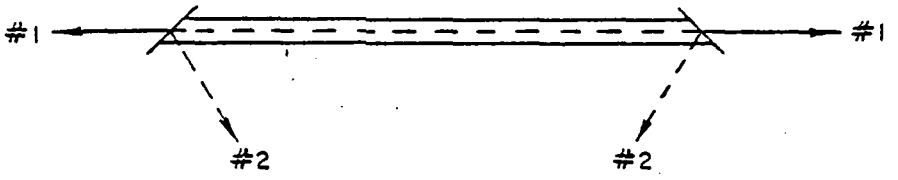


Angular divergence of laser beam

Fig (1)



(a) Parallel end windows



Main laser beam = #1 Stray beams = #2

(b) Anti-parallel end windows

Stray beams in gas lasers due to reflections
from Brewster angle windows

Fig (2)

Flash Lamps - Associated Hazards

The use of flash lamps for pumping lasers is widespread and historically is the oldest method of laser excitation. The considerations which follow discuss the present state of the art in flash lamps or optical pumping devices and indicates the hazards associated with their use. The term flash lamp is rather generic and to be more specific, the optical pumping methods to be considered here will be:

1. Conventional gas filled tubes, both linear and helical
2. Annular discharge tubes
3. Inductively coupled, theta pinch discharges
4. Exploding wires.

1. Conventional Flash Lamps.

These lamps, in both linear (including Pi geometry) and helical shapes are available commercially as very small lamps, one inch or less arc length, rated at a few joules to tubes with six-foot arc lengths, capable of loadings in excess of 200,000 joules. The major manufacturers of such tubes are listed in Table I.

Characteristically the larger tubes, six-inch arc length or longer, generate very intense bursts of optical radiation for time durations of the order of a few milliseconds. The actual maximum tube loading per inch arc length is a function of the discharge time and the tube bore size.

Figure I is a graph depicting the blow up ratings of various diameter, linear tubes and a derating guide for various operating conditions.

[illegible] Suppliers of High Energy Flash Lamps

* [illegible] Company
[illegible]

* [illegible] and Grier Inc.
[illegible]
Dixon, [illegible]

* [illegible] Inc.
[illegible]
[illegible]

[illegible]
[illegible]
[illegible]

[illegible] Inc.
[illegible]
[illegible]setts

[illegible]
[illegible]
[illegible]setts

* [illegible] 200,000 joule loading.

The light output from flash lamps has a spectral distribution governed by parameters such as:

1. Type of fill (i.e. Xe, A, O_2, He, etc.)
2. Fill pressure
3. Discharge characteristics (current density, discharge time, etc.)

An important consideration in the use of these lamps is the spectral conversion efficiency. This is a function of the above parameters and essentially tells how much of the input electrical energy is converted to optical radiation at various optical wavelengths. Generally the spectral distribution of the radiant energy is characterized by lines superimposed on a continuous background. There appears to be no coherent description which, in simple form, enables one to predetermine all the properties of a given flash lamp. Considerable evidence is given by Marshak (1) however and some general observations are given. The total conversion efficiency for the conversion of electrical energy to radiated optical energy in the 12,000 Å - 2,000 Å interval tends to a single limit independent of gas fill under special conditions. This conversion efficiency increases somewhat with the atomic weight (the reason most tubes are filled with Xenon) of the gas but is nearly the same for gas pressures on the order of 100 mm Hg, for tube radii of 6-12 mm and for the electric field across the tube about 100 volts per cm (1). As a general rule of thumb, the overall conversion efficiency in this case is about 50% - 60% of the input energy (29). The balance of the energy is lost in heating the tube, vaporizing parts of the tube and electrodes and heating in the external leads (1,3).

The spectral distribution of the emitted radiation covers the wavelength interval from below 12,000 Å to more than 2,000 Å and is determined largely by the maximum current density which flows during the discharge (1,2,4,5,6,7,9). At relatively low current densities, below 1,000 amps/cm^2, the spectral distribution is practically uniform across the entire spectrum from 3000 Å to 12,000 Å, with some prominance in the infrared. As the current density is increased to the region of 2000 amps/cm^2 the infrared begins to saturate. As the energy and current density in the discharge is increased the spectrum shifts towards the blue as shown in Figure II. The shift in spectral energy distribution continues further towards the violet with saturation of output in the visible region occurring in the vicinity of 6000 - 7000 amperes/cm^2 (7). Higher current densities have been achieved using preionizing techniques (double pulsing) (4,5,6). At current densities of 20,000 amps/cm^2 the spectral distribution is such that the ultraviolet (4500 Å and shorter) makes up nearly 20% of the output while the visible (4000 - 7000) is about 50% of the output and the infrared (7000 - 12,000 Å) about 30% (4,5,6). A problem that occurs repeatedly in the literature is that no one researcher shows the full spectral distribution of the flash lamps studied. The above figures must be regarded with some caution since they represent an approximate interpretation of the results of several investigators.

The largest linear tubes that are commercially available in the United States today have a loading capability of 200,000 joules. Helical tubes are also manufactured with input ratings up to 240,000 joules (see Table I). However the maximum tube ratings are given in terms of joules/inch of

various diameter tubes (Fig. I) with no limitation on length. It is thus quite possible to theoretically manufacture tubes of any energy rating desired. The practical limit in length appears dictated by the application. In the laser field this appears, at present, to be limited to tubes with an arc length of 1.8 meters (about 6 feet). In 1962 the Russians reported operation of linear tubes with an arc length of 1 meter at loadings in excess of 100,000 joules (1). Their continued work in the area of higher energy flash lamps does not seem to be in the open literature.

The most popular type of linear flash lamp is that represented by the EG&G FX-47, that is, a 13 mm bore, 16 cm arc length , 10,000 joules loading, Xenon filled tube. Typical pulse operation with this type flash tube is about 10,000 joule input for pulse times between 1 and 3 milliseconds. For this loading, the total radiation energy will be approximately 5,000 joules. Similarly a 200,000 joule tube will radiate about 100,000 joules in optical energy, based on a 50% conversion efficiency.

2. Annular Flash Lamps

Flash lamps based on a discharge in an annular section between ring electrodes have been used in flash photolysis (8) and laser pumping (7). Annular lamps have been compared to helical lamps by Keubler and Nelson (9) and to linear lamps by Church et al (7). A schematic drawing of an annular lamp is shown in Figure III. Kuebler reports efficiencies an order of magnitude below that of helical lamps when the outer wall of the annular lamp is opaque. Church, on the other hand, with a heavy coating of reflective magnesium oxide covering the outer wall of the lamp reports ruby laser pumping efficiencies of 0.5%, which is comparable to the efficiencies obtained with linear and helical flash lamps.

3. Theta Pinch Lamps

The recent application of the theta pinch discharge to laser pumping (10,11,12,13,14,15) has introduced a new concept of optical pumping. Primarily the theta pinch is an inductively coupled, electrodeless discharge of similar geometry to the annular tube, except that the current flows circumferentially, wherein relatively high energy electrons excite resonant atomic transitions which emit the radiations. In this procedure the conversion efficiency of pulse energy to radiated energy is very high and thus the temperature of the light source remains low. Since initial gas pressure is low, a few mm of Hg, and the atoms and ions do not gain appreciable kinetic energy, the shock affects for short discharge times is not a limiting factor on operation as it is in the case of the much higher pressure Xenon flash lamps. Thus much faster light flashes can be obtained.

Order of magnitude conversion efficiencies lie in the range 50% - 90% of the induced energy. However, due to leakage inductance effects no more than 50% of the stored electrical energy can be delivered to the tube. The overall efficiency of the theta pinch tube thus lies in the range 20% - 40% of the initially stored energy.

The output spectral distribution is reasonably flat across the entire optical region, in Argon, at initial stored energies up to 800 joules (14). There is little information concerning spectral distribution at other loadings and in other gases, but the suggestion has been advanced that the spectral distribution may be easily selected by proper choice of gas fill (13,14,15), and that the ultraviolet predominates at higher loadings.

Theta pinch discharges as short as 2 μsec have been used in laser pumping experiments with loadings up to 800 joules (10,11,12,14). These lamps were unshielded except for the current induction coil which was wound around the periphery of the tube. Theta pinch discharges have also been operated with 30 μsec rise times at 1,500,000 joule loading by Kolb and others (15). These large devices were extensively shielded to prevent the emitted radiation from entering the ambient atmosphere. This precaution was necessary due to the very high peak power radiation which, when absorbed by the air in the vicinity of the tube, created an intense shock-wave sufficient to destroy the tube. Kolb indicates that at loadings in excess of 1000 joules the spectral distribution should be enhanced significantly in the ultraviolet, determined mostly by the radiation from the fast electrons.

4. Exploding wires.

Various exploding wire light sources have been used in experimental laser pumping studies at discharge times as short as 8μseconds and loadings up to 16,000 joules per wire (16,27). The spectral conversion efficiency appears to be similar to that of Xenon flash lamps. The work of Church et al (16) seems to indicate that the exploding wire light source is not as well suited for laser pumping as the more conventional flash lamps.

Flash Lamp Hazards.

The most obvious hazards from the intense, transient radiation from flash lamps and similar devices are irreversible and reversible damage to the eye (17). A worst case analysis, under certain assumptions, can

indicate at least the minimum safe viewing distance at which irreversible retinal damage and reversible external radiation effects may be minimal. Other flash lamp hazards include ultraviolet erythema and possible carcinogenic effects, X-ray production, electrical shock hazards, flash lamp explosion and the possible production of poisonous gasses and vapors.

Optical Radiation Hazards.

Linear flash lamps, and to a good approximation, helical lamps are essentially line radiation sources. Their radiant power distribution does not follow the ordinary distribution for a point light source. For a line source the radiation pattern is essentially toroidal (17) as shown in Figure IV. At a distance R from the lamp (R greater than the largest lamp dimension) the radiation energy varies from a minimum at $\theta = 90^{\circ}$. Thus at R, the surface energy density, for a lamp energy output of E_o joules is:

$$E_{R,o} = \frac{1}{\pi^2 R^2} E_o$$

with the Lambertian angular dependence at any θ,

$$E_{R,\theta} = E_{R,0} \cos \theta$$

The optical radiation hazards from intense transient light sources can be best evaluated on a worst case basis. The worst case conditions to be assumed here are:

1. The lamp is unshielded.

2. A potential observer is located perpendicular to the tube axis at the mid-point of the tube, that is $\theta = 0^{o}$

3. The observer is completely dark adapted with a pupil area of 1 cm^2 (for retinal damage)

4. For light focused by the eye the retinal spot size is 100 sq. microns.

5. The threshold for retinal damage is 1 joule/cm^2 and that this value holds for flash times from a few microseconds to a few milliseconds *

6. The observer has normal, white untanned skin (skin erythema hazard)

7. The threshold of UV skin erythemal reaction is assumed as 0.05 joules/cm^2 **

8. The threshold for the onset of photoinduced ultraviolet conjunctivitis is assumed as 0.07 joules/cm^2 ***

Under the above approximations the threshold hazard level for retinal damage, conjunctivitis and skin erythema can be given numerically in relation to flash lamp input energies. As an additional approximation the radiation from the flash lamps will be considered to be related to the flash lamp loading in the following manner.

1. Radiation taking part in retinal damage is approximately 50% of input.

2. UV radiation taking part in conjunctiva and skin interaction is approximately 20% of input.

---

* From the work of Ham, Geeraets, et al., summarized by Christner et al (17).

** This threshold has been determined at the maximum spectral sensitivity for a wavelength of 2967 Å (19,20). This value will be assumed to hold for all UV radiation in the worst case approximation.

*** This value is assumed from the work of Kinsey (21) who showed humans to be twice as sensitive as rabbits and from the threshold value discussed in Duke-Elder (22) for rabbits at 2880 Å - this value will be used, although its validity is questionable.

Threshold hazard distances can be plotted now as a function of flash lamp inputs for these three hazards. To repeat, the surface threshold energy densities are:

1. Retinal burn: 10^{-6} joules/cm^2 at pupil (1 joule/cm^2 at retinal spot size of 100 sq. microns, 1 cm^2 pupil area).
2. Conjunctivitis: 7×10^{-2} joules/cm^2 at corneal surface
3. Skin erythema: 5×10^{-2} joules/cm^2 at skin surface.

The retinal damage threshold versus distance is shown in Figure V. For example, the most commonly used flash lamp (10,000 joule loading) would have a threshold for damage distance of about 220 meters if flashed with no shielding.

The skin and exterior eye thresholds for photo reaction thresholds are shown in Figure VI. For the 10,000 joule lamp, mentioned before, perceptible skin erythema or conjunctiva effects could occur at distances of about one half meter.

Thresholds for infrared damage to the cornea, conjunctiva, iris and lens should be considered apart from the integrated intensity of ultraviolet effects. There does not however appear to be any literature detailing the effects at the longer wavelengths (7000 - 8000 Å and longer) with respects to other established hazard thresholds.

It must be pointed out that in almost all operating laser systems, the optical pumping lamps are enclosed in a shielded cavity. The cavity is designed specifically to collect as much pumping radiation as possible and deliver ti to the laser rod. Cavities can be made light tight, but

in practice some pumping radiation usually leaks outside the cavity. The worst case analysis above serves not only as a guide to viewing exposed or unenclosed flash lamps, but also as a reinforce ment to the suggestion of "don't look" when operating optically pumped lasers.

Other Flash Lamp Hazards

The possibility of ionizing X-rays being produced under normal flash lamp operation is very low. The electrons in the discharge can never attain X-ray energies for the relativly long pulse times ordinarily used and the relatively low voltages (3,000 - 5,000 volts) it seems natural to expect the presence of X-rays. It appears as if this is not the case however. Electron energy distributions in plasmas are discussed in general by Loeb (23) and in the case of theta pinches by Kolb (15). On a classical, kinetic theory basis one would expect the highest electron energies to appear in the lower pressure discharge, namely the theta pinch. The data of Loeb confirms this. A personal communication with Mr. H.W. Klauer of Kemlite Laboratories Inc., indicates that in Xenon flash lamps there is no danger of X-rays below 15,000 volts operating potentials. However, above 15,000 - 20,000 volts he claims there may be a danger of "soft gamma radiation." Kolb's calculations indicate maximum electron temperatures (energies) of less than 100 electron volts in the theta pinch and the work of Feldman seems to be similar (14). Our own calculations based on a strictly kinetic theory model show maximum electron energies to be of similar order of magnitude in Xenon discharges at 20,000 and 40,000 volts. Experimental x-ray measurements by Vitkovitsky et al (24) on exploding wires at driving voltages up to 320,000 volts

showed no high energy X-ray production. Maximum X-rays observed in the exploding wire studies were of the order of 5,000 electron volts (λ = 2.5 Å). X-rays at this wavelength are attenuated rapidly by quartz and glass and have a relatively short path length in air. Thus, although no study of possible X-ray production in flash lamps has been undertaken, the evidence clearly indicates that the X-ray hazard from flash lamps themselves is minimal. The major X-ray hazard source associated with flash tubes appears to be in the high vacuum rectifier tubes used in the capacitor charging power supplies, for in high vacuum tubes the electrons can reach an energy, in electron volts, equal to the peak voltage across the tube, as is the case in ordinary X-ray tubes.

Other potential hazards in flash lamps include:

1. Flashlamp failure produces a sharp acoustical report and flying quartz fragments which can be very hazardous to unprotected personnel in the immediate area.

2. Poisonous gases can accumulate in the laboratory if not properly ventilated. The generation of O_3 by ultraviolet irradiation is well known and the decomposition of CO_2 to CO under flash photolysis has been shown (25).

3. The possibility of neutron production in theta pinch discharges due to low atomic weight impurities such as hydrogen, deuterium and helium has not been fully explored.

4. Flash lamps operate at several kilovolts or more so that proper insulation and good grounding procedures should be followed.

Potential Flash Tube Developments

Over the next three years no major advance in the state of the art of flash lamps is to be expected. Present research in the doping of Xenon flash lamp with various elements will probably be continued in an attempt to modify the output spectral characteristics of the flash (7).

There is, as stated previously, no great technical problem in producing higher energy flash lamps, the arc length and bore size are just increased. However the trend in laser design seems to be in the utilization of oscillator-amplifier arrays in which, at present, as many as ten flash lamps modules have been used to create haigh energy laser pulses (26). This would seem to indicate that much larger linear lamps will not probably be produced.

Helical flash lamps are expected to be produced with capabilities up to 500000 joules in the next three years according to Mr. H.W. Klauer of Kemlite Laboratories, Inc.

The application of high energy theta pinch discharges to laser pumping does not appear very extensive. Due to the enhanced ultraviolet output at higher energies and the low overall efficiencies, this source does not seem as potentially useful for efficient laser work (personal communication with Dr. Buser, Army Signal Corps Laboratories, Fort Monmouth, New Jersey).

References

1. Marchak, I.S., "Strong-Current Pulse (Spark) Discharges in Gas, Used in Pulsed Light Sources," Soviet Physics Uspekhi, 5:478, 1962.
2. Gonce, J.H., and Newell, B., "Xenon Flashtube Spectra FX-47 and FX-47A," EG&G Technical Memorandum No. B-430, 1963.
3. Beeck, A., Erickson, R., Barnes, F., "Design and Operation of Xenon Flash Tubes" J. Appl. Physics, 34:2115, 1963.
4. Emmett, J.L., Schawlow, A.L., "Enhanced Ultraviolet Output from Double-Pulsed Flash Lamps," Applied Phys. Lett. 2:11, 1963.
5. Gandy, H.W., et al, "Persistent Enhanced UV Radiation from Double-Pulsed Flash Lamps," Appl. Phys. Lett., 4:11, 1964.
6. Park, S.W., "Double Pulse Technique for FX-47 A," EG&G Report, June 30, 1964.
7. Church, C.H., et al "High Energy Coaxial Laser Pumps," Laser Flash Lamp Conference, Stanford Research Insitute, February 1964.
8. Claesson, S., Lindquist, L., Arkiv för Kemi 12:1, 1958.
9. Kuebler, N.A., Nelson, L S., "Radiant energies and Irradiances of Capacitor Discharge Lamps," J O.S.A., 51:1411, 1961
10. Brandewie, R.A., Hitt, J.S., and Feldman, J.M., "Plasma Pinch Excitation of A Ruby Laser," J. Appl. Phys. 34:3415, 1963.
11. Haswell, W.T., Hitt, J.S., Feldman, J.M., "A High Repetition Rate Laser System," Proc. IEEE, 52:93, 1964.
12. Hitt, J.S., Feldman, J.M., "Parrallel Theta-Pinch Pumping of a Laser Oscillator-Amplifier," Proc. IEEE, 52:616, 1964.

13. Aisenberg, et al, J. Appl. Phys, 25:3625, 1964.

14. Feldman, J.M., "Theta Pinch Lamp," Laser Flash Lamp Conference, Stanford Research Institute, 1964.

15. Kolb, A.C., "NRL Research in Theta Pinch and Double Pulsed Lamps," Laser Flash Lamp Conference, Stanford Research Institute, 1964.

16. Church, C.H., et al, "Optical Pumping of Lasers Using Exploding Wires," Westinghouse Research Laboratories Scientific Paper 62-112-259PL.

17. Christner, C.R., et al, "State-of-the-Art Study on Visual Impairment by High-intensity Flash of Visible, Infrared, or Ultraviolet Light," Report No. BAT-171-9, Battelle Memorial Institute, Jan. 1965.

18. Uhl, R.J., M.I T., Servomechanism Lab. Memorandum No. 7668-TM-3, 1958.

19. Luckiesh, M., Taylor, A.A., "Erythemal and Tanning Effectiveness of Ultraviolet Energy," G.E. Review, P. 274, June 1939.

20. Coblentz, W.W., Stair, R., Hogue, J.M., "The Spectral Erythemic Reaction of Untanned Human Skin to Ultraviolet Radiation," U.S. Bureau of Stand. J. of Res., 8:541, 1932.

21. Kinsey, V.E., Cogan, D.G., and Drinker, P., "Measuring Eye Flash from Arc Welding," JAMA, 123:7, 403, 1943.

22. Duke-Elder, S., Textbook of Ophthalmology, Vol VI, C.V. Mosby Co., St. Louis, Mo., 1954.

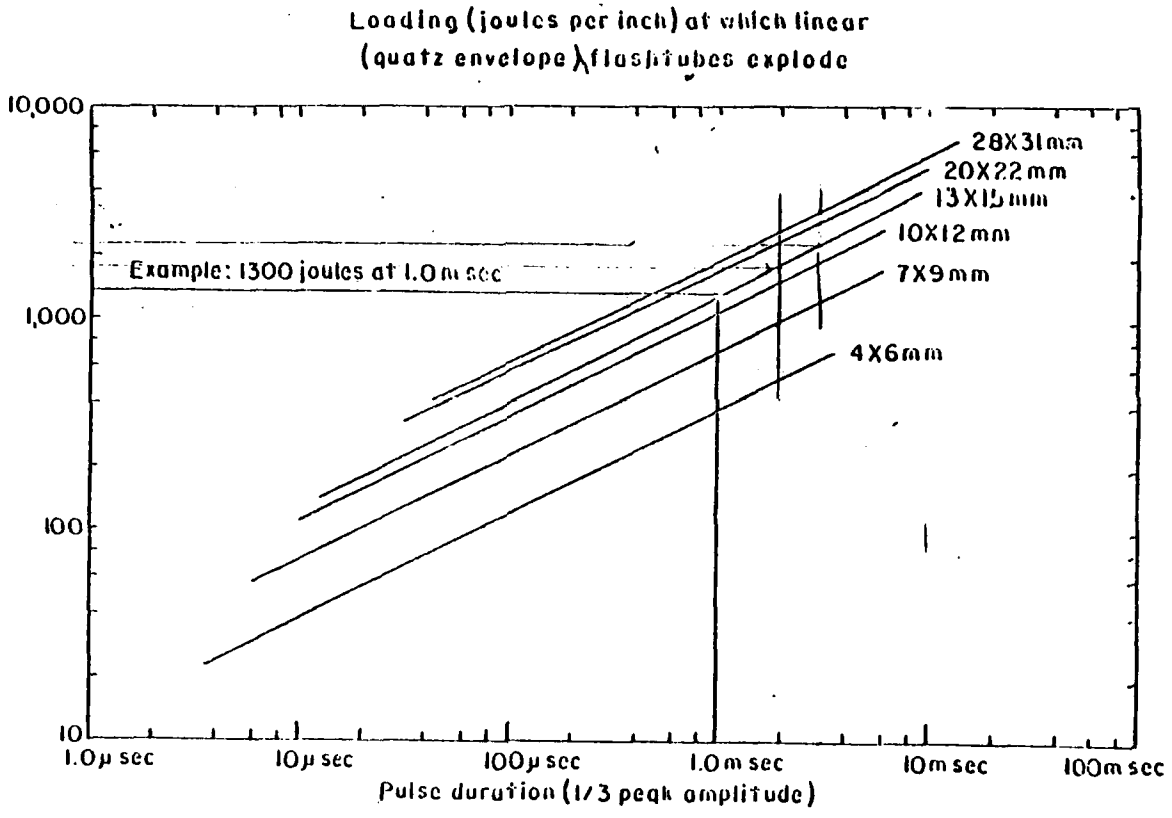
23. Loeb, L.B., Basic Processes of Gaseous Electronic, Univ. Calif Press, 1960.

24. Vitkovitsky, I.M., et al,"Exploding Wires as A Source of X-rays," Exploding Wires, Vol 2,(N.Y.:Plenum Press, 1962) ed.by W.G. Chase and H.K Moore.

25. Bortner, M.H., "Actinometric Determination of the Dissociation of Carbon Dioxide by a Single Flash," JOSA, 50:172, 1959.

26. Dearman, J.R., et al, "Consideration in the Design of High Energy Neodymium Lasers For A Surgical Device," First Annual Biomedical Laser Conference, Boston, June 1965.

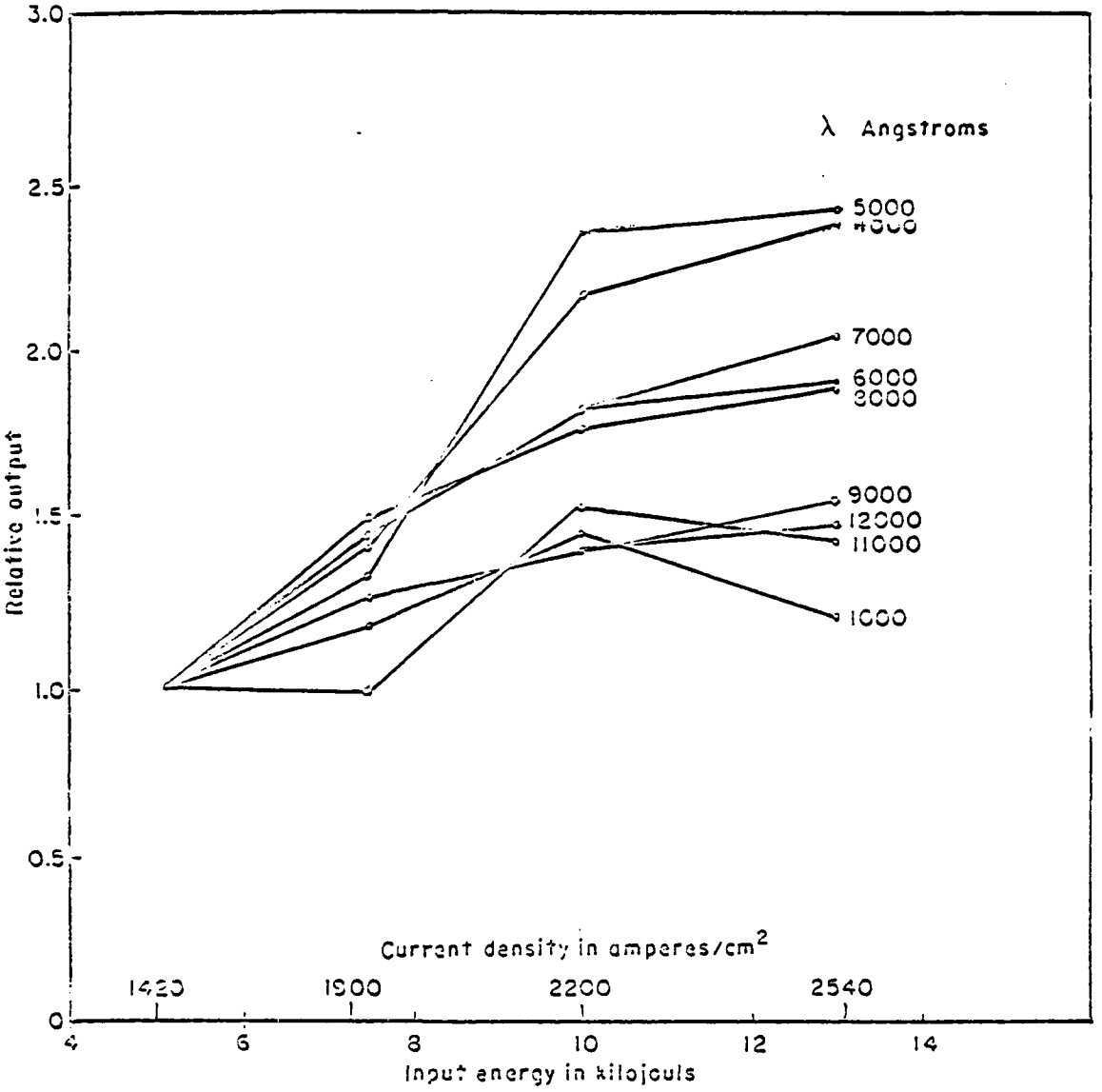
27. Clark, G.L., Chase, N.C., and Moore, H.R., "High Intensity Pump for Optical Lasers," Technical Documentary Report No. ASD-TDR-63-651, WPAFB, Aug. 1963.



Derating Guide: For free air operation and reliable life use 70% of chart value, for cavity operation, use 40%

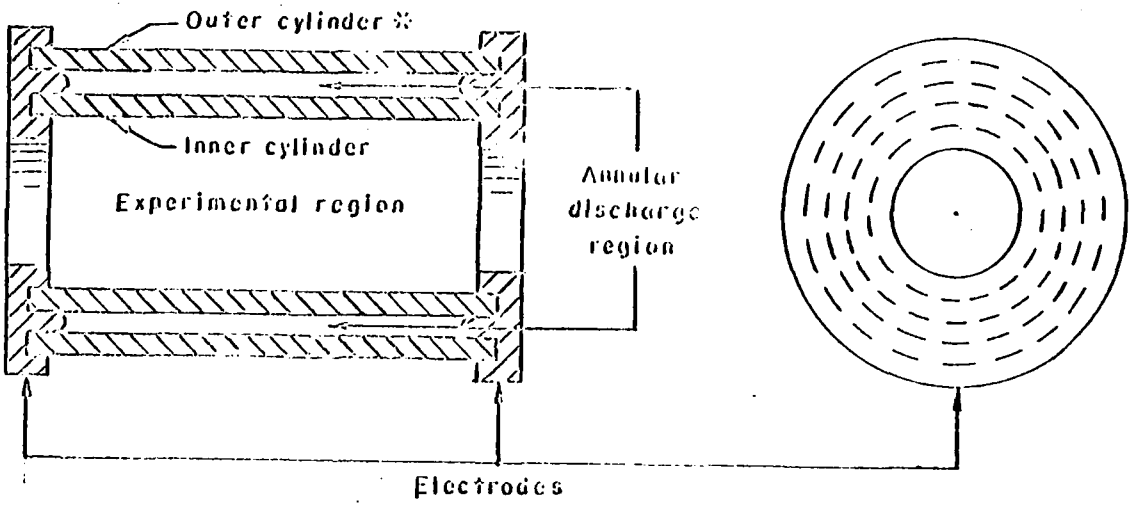
from EG&G Data Sheet 1002

Fig I



Relative output of EG&G FX-47A Xenon Filled (300 mm HG) flash lamps at various wavelength versus input energy and current density (3.0 msec. pulse).
From Gonez and Newell

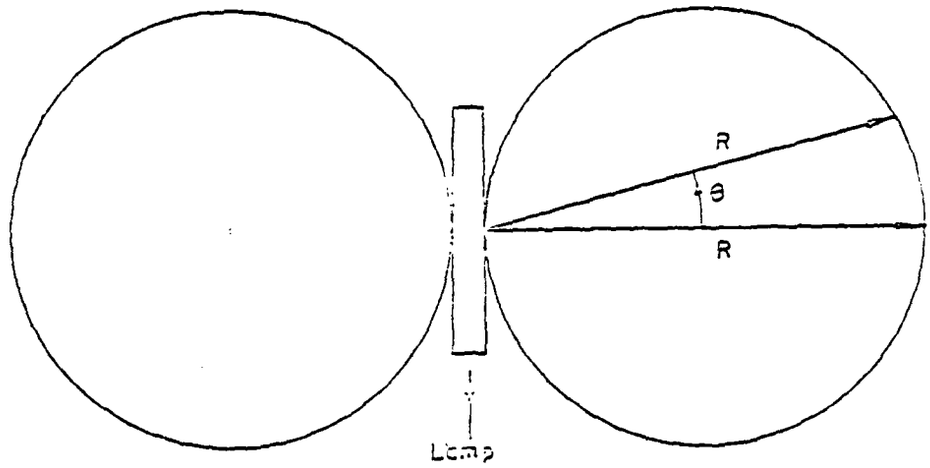
(Fig 9)



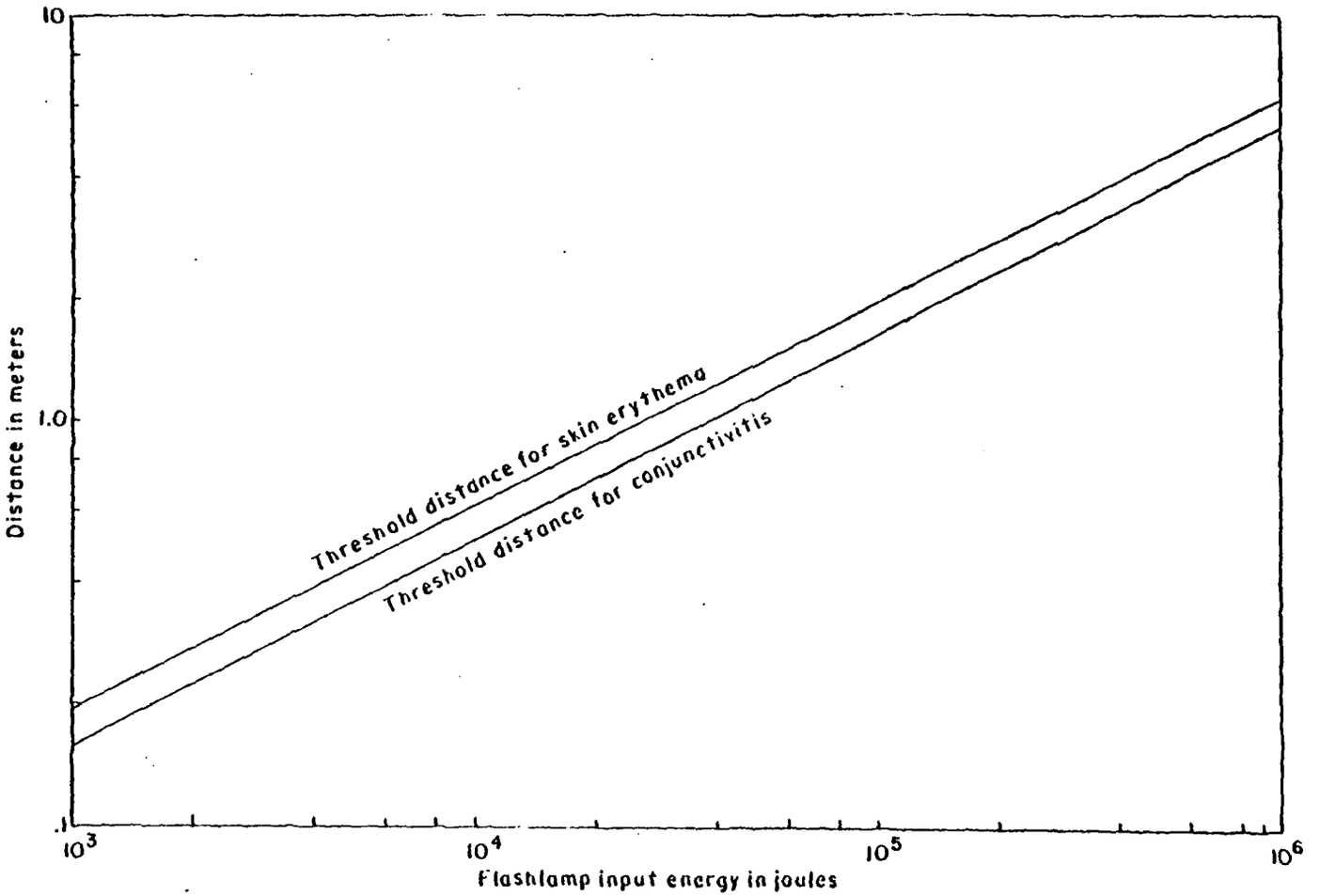
*Outer cylinder usually opaque or reflecting

Annular Flash Lamp Geometry

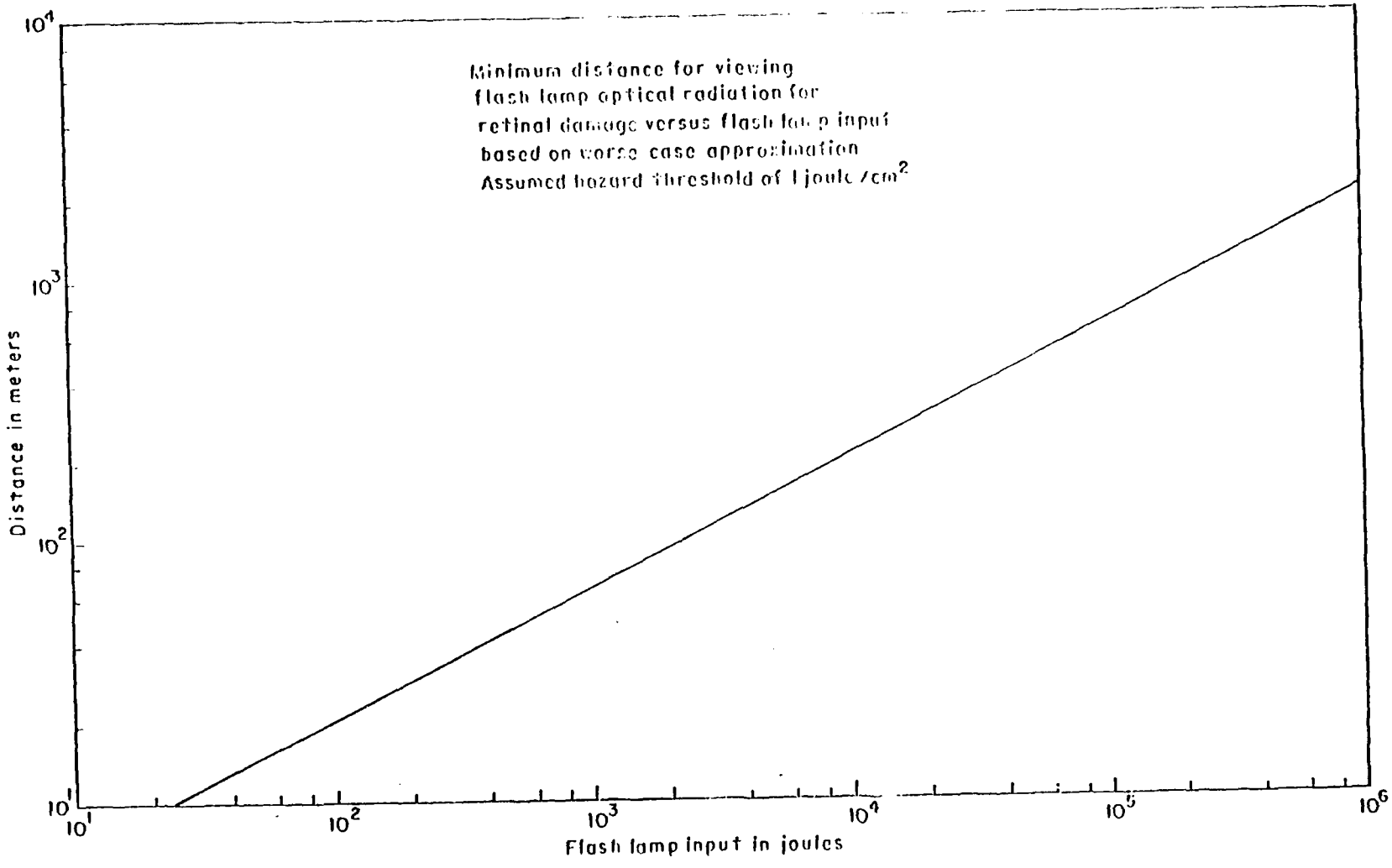
(Fig. 2)



Radiation pattern from linear flash lamp where R is large compared to largest lamp dimension



Threshold distances for skin and corneal photo reactions from flash lamp radiation.


Minimum distance for viewing
flash lamp optical radiation for
retinal damage versus flash lamp input
based on worse case approximation
Assumed hazard threshold of 1 joule/cm²
Distance in meters
10⁴
10³
10²
10¹
10¹
10²
10³
10⁴
10⁵
10⁶
Flash lamp input in joules

Electrical Hazards

In the laboratory, electricity is potentially one of the most dangerous commodities. Accidental electric shock is at best annoying and at worst fatal. The small number of electrical fatalities is probably due to the general awareness that electricity is inherently dangerous, and to the concerted effort in design and construction of electrical system. The electric shock hazards of both high-voltage and low-voltage circiuts are derived from known effects of electric currents as determined from low-voltage experiments. Data on currents large enough to cause sudden death in man must be extrapolated from animal studies (1). Although translation of results obtained on animals to man is conjectural, in many instances analysis of human accidents has permitted correlations to be made (2).

A summary of possible quantitative effects of electric currents on man is given in Table I (2). The values of "Electric Current" necessary to cause ventricular filrillation in man were derived from tests made on animals and are what Dalziel termed "best estimates."

It is important to note from Table I the levels at which electric currents are hazardous. These levels are very low. When one comes in contact with a hazardous situation, a difference in potential exists across the path through the person. This difference in potential must be related to the current given in the table. The proportionality factor is the resistance of the body, which, however, is not a constant. Pitre and Dahlberg (3) have made studies on low voltage hazards. Their studies show that voltages far below

TABLE I

Quantitative Effects of Electric Current on Man

Effect	Milliamperes (thousandths of an ampere)					
			Alternating Current RMS Values			
	Direct Current		60 Cycle		10,000 Cycles	
	Men	Women	Men	Women	Men	Women
No sensation on hand	1	0.6	0.4	0.3	7	5
Slight tingling. Perception threshold	5.2	3.5	1.1	0.7	12	8
Shock -- not painful and muscular control not lost	9	6	1.8	1.2	17	11
Painful shock -- painful but muscular control not lost	62	41	9	6	55	37
Painful shock -- let-go threshold	76	51	16.0	10.5	75	50
Painful and severe shock -- muscular contractions, breathing difficult	90	60	23	15	94	63
Possible ventricular fibrillation from short shocks:						
Shock duration 0.03 sec.	1300	1300	1000	1000	1100	1100
Shock duration 3.0 sec.	500	500	100	100	500	500
Ventricular fibrillation -- certain death	Multiply values immediately above by 2 3/4. To be lethal, short shocks must occur during susceptible phase of heart cycle					
Possible ventricular fibrillation from impulse shocks:						
DC short shocks and surge discharges	27.0 watt-seconds					
Power-frequency short shocks and low-frequency oscillatory discharges	13.5 watt-seconds					

that produced by the common 110 volt supply have caused fatal electrical accidents. Fatal shock from low voltages usually occur under conditions which are conducive to lowering skin resistance at the points of contact.

Working conditions which are particularly hazardous on low voltage exposures include:

1. wet, humid or damp locations
2. High temperatures, since they are likely to cause perspiration
3. locations where the individual is exposed to grounded surfaces.

Many of the hazards associated with electrical circuits can be alleviated by the use of proper precautions. Some basic considerations are:

1. the proper selection of equipment for the type of exposure for which it is to be used.
2. Installation of the equipment in accordance with established safety standards.
3. Inspections at frequent intervals by competent personnel to develop a proper maintenance program.
4. Maintenance to keep the equipment and circuits in safe operating conditions.

The electrical shock hazard can be greatly reduced by any of the following protective methods, or by a combination of them:

1. Effective grounding of non-current carrying parts.
2. Insulation of exposed metallic surfaces
3. Electrically isolating the operators.
4. Using voltage low enough to minimize the shock hazard.

Grounding of non-current-carrying parts of equipment is carried out to protect personnel against electric shock from metal enclosures or frames of motors, transformers, switchboards, portable tools and lamps, etc., in case such parts accidentally come in contact with live parts of the circuit. Where protective grounding is found to be impractical, insulation of the non-current-carrying exposed metal parts may be employed. Such insulation should be made to withstand the abuse expected under working conditions and should be made resistant to oil, grease, and moisture.

Where neither grounding nor insulation of non-current-carrying parts are employed, protection in hazardous exposures can be accomplished by insulating the operator by the use of rubber mats or shields, or by wooden platforms or barriers. The purpose is to prevent any return path for the current through the body of the operator.

Those charged with the responsibility of the safety of the individuals must carefully survey the electrical exposure, employ proper methods of control and educate the workers regarding the hazards involved. Safety personnal should be guided in their responsibilities by regualtions set down by the National Electric Code (4).

Whitaker (5), and Inship and Watson (6) historically follow the development of the gounding requirements of the National Electric Code along with the reasons for each. They also emphasize that the principle method for grounding portable equipment is through the line-cord which includes a grounding conductor with a 3-pole American Standard attachment

plug cap which has a pair of parallel blades and a round or half-round grounding blade. There should also be a receptable which establishes a ground connection to the same electrode as used for the system ground at the service entrance. Alternatively the same cap and receptable may be used with a cable that has an armor or metal enclosure of the circuit conductors with the enclosure being used as the grounding-conductor and attached to the grounding blade. A third method is also mentioned briefly. This consists of a separate grounding conductor, as a bare or insulated flexible wire or strap not in the supply cord, used only when part of approved equipment or by special permission of the enforcing authority. Consequently, in laser systems the first or second method should be used on all equipment to provide adequate grounding.

Grounding does not always insure the greatest measure of protection to personnel and, under certain conditions actually may increase the hazard. The presence of well-grounded metal cabinets as opposed to isolated metal contributes to the establishment of conditions necessary to cause shock to persons who touch live current-carrying parts. This may or may not have a particular application to a particular laser system. An individual system may require an isolated system but in general the grounded system would be preferable because in this case it affords better protection for the operators.

Grounding is not intended as a substitute for proper spacings, adequate dielectric strength tests, or the use of proper insulating materials, but is a supplemental safety measure to all these features.

Heddesheimer (7) discussed plant and building grounds as well as equipment grounds. He lists the grounded Y system as the safest for both equipment and personnel. As for the establishment of grounds, all steel frames of buildings must be adequately grounded. As a minimum a ground bus of not less than No 4.0 American Wire gauge copper should be run around the periphery of the building, connected to each outside building column, and connected to earth at not greater than at 200 foot intervals.

Each water main entering the building, if suitable for use as a ground, should be connected to the ground bus at two or more points. In large buildings the ground bus should be extended to connect to an adequate number of internal building columns.

The connection to earth and the associated problems of keeping the "to earth" resistance low are carefully covered by Heddesheimer (7). Following proper grounding of the laser system, attention can be directed to another electrical hazard. This is the complete and safe discharge of any and all capacitor banks. The most dangerous situation of all exists when the laswer system has been operating satisfactorily.. When the laser has fired and the capacitor bank has dumped most of its charge, the capacitor bank is still unsafe electrically. Referring to Table I, it can be seen that it is possible for as little as 27 watt-seconds (joules) to be lethal. It is quite probable that after normal firing of the laser much more than this amount of energy remains stored in the capacitor bank. Since insulation resistance of typical capacitors is extremely high, capacitors may retain stored charges for days (15). As a safety feature some method should be provided to completely and safely discharge the bank

manually as well as automatically to the point where no energy remains stored, Calculations are carried out in the following part of this report to indicate the necessity of this provision.

Dalziel (8) discusses the hazards of impulse currents. He attempts to define shock intensity in terms of energy and then projects a hypothesis that for short shocks, energy is the fundamental criteria, with current magnitude, duration and total charge being related quantities of secondary importance.

These impulse studies were carried out both with oscillatory and non-oscillatory discharges. It was found that the tolerance to surge discharges was greater than for oscillatory discharges. This was based on studies to determine the currents which are just perceptible on the hands, where the currents for pure direct current and 60-cycle alternating current are in the ratio of 4.8 to 1 (10,11).

A comparison of impulse type shocks as opposed to the non-impulse type is not readily apparent from the literature.

As an example of the hazard due to an impulse shock, the stored energy ($E = \frac{CV2}{2}$) is plotted as a parameter on a graph of capacity of the bank versus the potential across the bank. The curves are straight lines when plotted on log-log scales. According to the data given by Dalziel (2,8), any condition to the right or above the 27 joule line (the solid line) may be considered extremely dangerous. It can be seen that as the capacitor banks increase in size with a small potential across the bank a dangerous situation can exist. For example for a 100,000 microfarad bank 25 volts could be

fatal. Smaller banks can present significantly hazardous conditions. A ruby laser that will deliver 100 joules and is 1% efficient will require 10,000 joules of pumping energy. If the bank is charged to 3,000 volts then 2,200μf will be required. From the graph one can see that corresponding to 2200 uf, 160 volts is extremely dangerous. If this laser system, following firing, does not discharge to significantly less than this value it must be considered dangerous. This will probably not be the case; 160 volts or more are left on the capacitor bank, and it will remain lethal and must be discharged previous to contact.

The high-voltage energy storage capacitors chosen for laser use should be those designed specifically for discharge applications which customarily involve varying cycles of intermittent duty. These capacitors are usually designed with a higher dielectric stress than is employed for conventional d.c. capacitors (15). In addition, provision is made to handle the high discharge current. The iesign also inclues minimization of self-inductance to assure short discharge time.

Since most high energy storage capacitors, except the aroclors (12) (which are also known as Askarels and are chlorinated diphenyls) are inflammable, fire safety facilities near large capacitor banks should be provided. When energy storage capacitors fail, resulting large currents may cause heating followed by combustion. Consequently safety circuitry designed to remove or monitor faulty capacitors are available.(12) and should be employed. Since most fuses would add too much inductance to the system, special fuses must be used in conjunction with protective

circuitry. Two such fuses are the silver sand current limiting type and the exploding wire fuse. When particular safety circuitry is chosen it should be kept in mind that major bus faults or failures in other parts of the circuitry may cause undersized fuse operation. This can be prevented by strategic placement of current limiting resistors. As a substitute for fuses small lengths of transmission lines with destruction levels below that contained in the unfaulted capacitor may be placed between each capacitor and a common bus. A separate transmission line should be run from each capacitor. Upon failure of a given capacitor, all the energy in the remaining capacitors will discharge through the transmission line associated with the faulty capacitors and destroy that transmission line. This will remove the faulted capacitor from the circuit, and permit operation with reduced capacity. However, it is uncertain whether this is advantageous and disadvantageous from a safety point of view.

An alternate method of fault protection is a method which monitors the internal pressure of individual capacitors and relays this information to a control panel. The impending fault may then be removed either manually or automatically.

The life expectancy or number of charge-discharge cycles of rapid discharge capacitors depends strongly on the operating conditions (12, 13, 14). An increase in voltage to as little as 120% of rated voltage may drop the life expectancy to 5% of its original value. The discharge time or ringing frequency also strongly effects capacitor lifetime. When the ringing frequency also strongly effects capacitor lifetime. When the ringing frequency is increased from 10^5 to 10^6 cps the life expectancy

decreases by an order of magnitude. The life expectancy is also affected by the amount of time the capacitor has been holding a charge, the degree of voltage reversal and the ambient temperatures. In light of such extreme conditions it is very advisable to stay well within the equipment ratings.

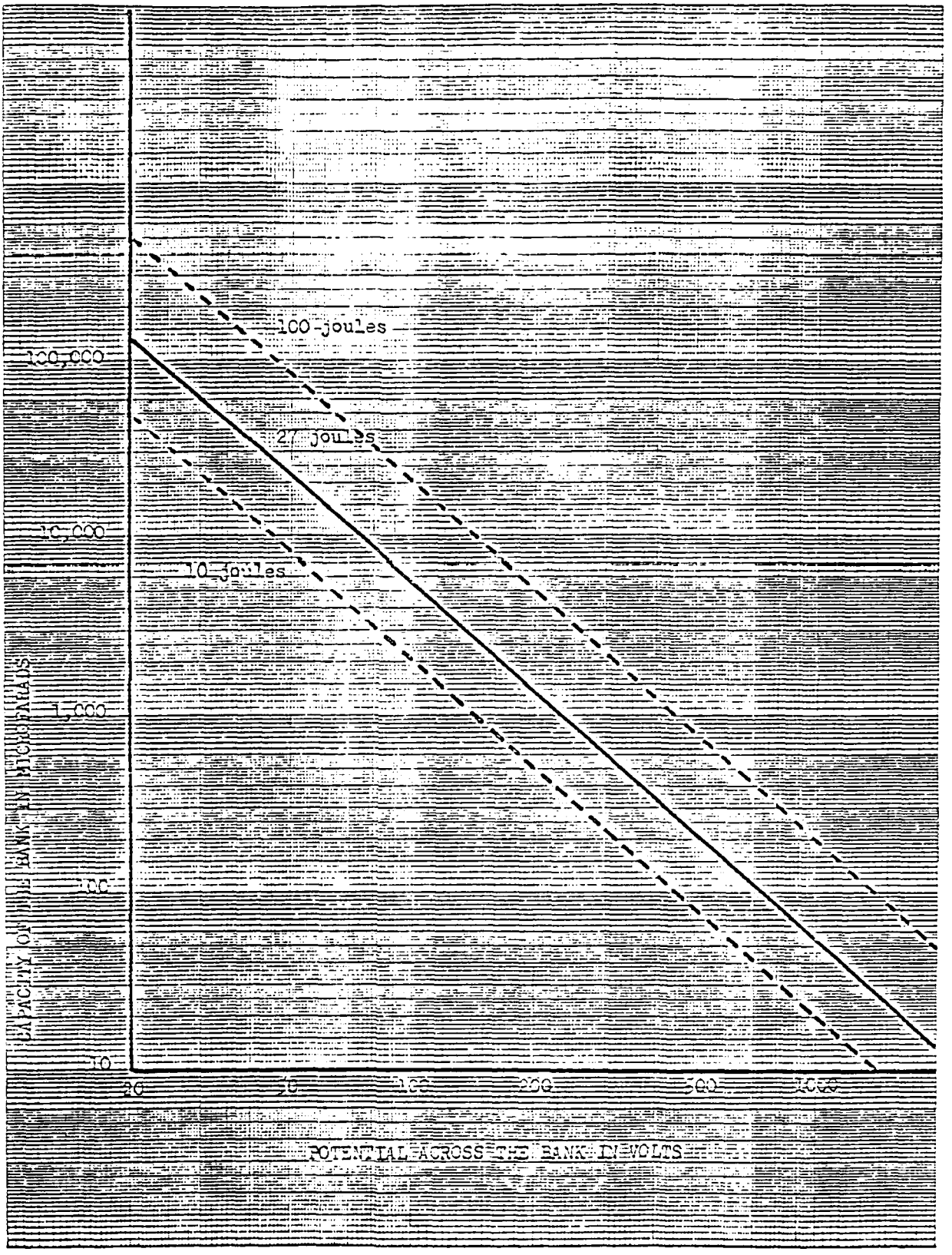
The capacitor life expectancy, for high energy storage capacitors quoted by manufacturers varies from 10^3 to 10^5 operations. This figure must be considered in light of the extreme variation in life expectancy under operational conditions (12,13,14).

Another item that is particularly prone to present safety problems are the high voltage current carrying cables. These cables should be placed so that accidental contact with them cannot occur.

In addition special consideration should be given to the choice of original cables. The cable chosen should be specified as corona free in addition to the adequate dielectric strength for the laser with which it is to be used. This will facilitate future field testing. Although actual observation of the cable and continuity tests are very important, the single most important test is the "hi-pot" or dielectric strength test. These tests can be made with readily available testing apparatus or by the cable manufacturers testing facility. The dielectric tests will give an indication of the present strength of the insulation and the corona test will test for the presence of the corona. If present, this corona causes ozone which is highly corrosive to some dielectrics and may cause ultimate failure of the cable. To be on the safe side, a cable that shows the presence of corona should be replaced. (*)

No discussion of electrical shock would be complete without mention of rescue and resiscitation for victims of serious electric shock accidents.

First aid should be restricted to minimal, essential manipulation of the patient as required for arrest of hemorrhage, coverage of the affected region with sterile gauze, and immobilization of the affected region, particularly in the event of fracture following electric shock. In the case of respiratory arrest, mouth to mouth artificial respiration should be immediately begun, and continued until medical attention is obtained. The equipment, techniques and knowledge necessary to apply controlled counter-shock in the short period available if ventricular fibrillation occurs and to attempt pacemaking is not available in the field. Consideration can be given to maintenance of cardiac output by external message. The literature in this field is in universal agreement that the application of artificial respiration, if the victim is not breathing or if he appears not to be breathing, should be continued without interruption until he revives or until he is pronounced dead by a physician. All accidents should receive immediate medical attention. A general physician, on call at all times, should be available for accidents. His telephone number should be posted in the laboratory.



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REFERENCES

1. Ferris, L.P., B.G. King, P.W. Spence, H.B. Williams: "Effect of Electric Shock on the Heart", Electrical Engineering 55:498, 1936.
2. Dalziel, C.F.: "Effects of Electric Shock on Man", AIEE transactions 75, July 1956.
3. Pitre, M.J., S. Dahlberg: "Low Voltage Hazards", Safety Engineering 90:22, 1945.
4. National Electrical Code, National Electrical Contractors Association, New York, N.Y.
5. Whitaker, H.B.: "Safety Aspects of Grounding Portable Electrical Equipment", AIEE transactions 71:897, 1952.
6. Inskip, L.S., H.H. Watson, "Grounding of Portable Electrical Equipment" AIEE transactions 74:286, 1955.
7. Heddesheimen, H.E.: "A Compedium of Grounding Techniques for Personnel and Equipment Protections", AIEE transactions 77:1225, 1958.
8. Dalziel, C.F.: "A Study of the Hazards of Impulse Currents", AIEE transactions 72:1032, 1953.
9. Dalziel, C.F., T.H. Mansfield" "Effect of Frequency on Perception Currents", AIEE transactions 69:1162, 1950.
10. Dalziel, C.F., E. Ogden, C. Abbott: "Effect of Frequency on Let Go Currents", AIEE transactions 62:745, 1943.
11. Dalziel, C.F.: "Effect of Wave Form on Let Go Currents", AIEE transactions 62:739, 1943.
12. "Energy Storage and Laser Capacitors", Cornell-Dublier Electronics Bulletin, Class 210.30.
13. "Energy Discharge Capacitors", Sangamo Electric Company, Bulletin TSC-208.

14. "Energy Storage Capacitors", General Electric Company.

15. "High-Voltage Energy Storage Capacitors for Laser, Photoflash, and Allied Applications", Sprague Engineering Bulletin, No. 2146.

(*) Telephone conversation with Mr. Paul Cardello, Boston Insulated Wire and Cable Co., 65 Bay street, Dorchester, Massachusetts.

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BIOLOGICAL EFFECTS OF LASER RADIATION. VOLUME I. REVIEW OF THE --ETC(U)
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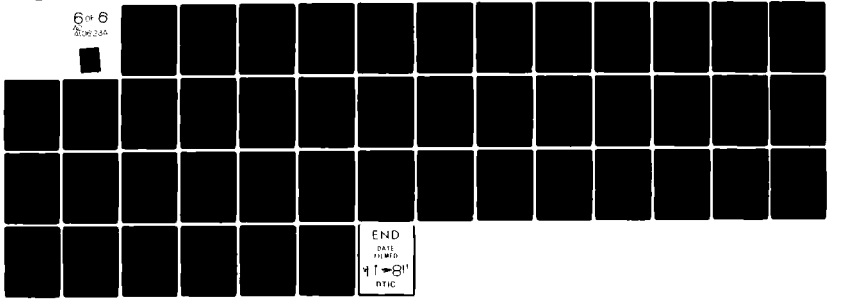

A D D E N D U M

ATMOSPHERIC SCATTERING OF LASER RADIATION AND ASSOCIATED HAZARDS

General method of approach

Atmospheric Scattering: Introduction

A laser transmitter employed for radar or communications normally emits a highly-collimated beam (typically 10^{-5} - 10^{-4} radians) with a beam diameter ranging from 1 millimeter to possibly 1 meter. Peak powers range from 10^{+9} to 10^{-3} watts. If the laser is to be useful, it must operate in a relatively transparent atmosphere. Under optimum atmospheric conditions, the attenuation of the beam as it passes through the atmosphere is exceedingly small.

Various effects lead to beam attenuation. Even in a clear, dry atmosphere, the beam is deflected and attenuated by turbulent motion of the atmosphere. As with most of the atmospheric scattering processes, the principal scattering direction is forward. A second process effective even in clear, dry atmospheres is molecular scattering. This process is strongly wavelength-dependent and is also a function of the atmospheric

composition. This process is of particular interest in the case of the CO_2 laser where the CO_2 in the atmosphere, especially urban atmospheres, leads to a substantial attenuation coefficient.

When the atmosphere is contaminated with aerosols and solid matter, particulate scattering becomes important. With the densities of particles found even in a moderate fog, each scattering event is independent.* Consequently, one can describe the scattering by a suitable average over the scattering particles. Obtaining such averages is an extremely complicated operation except in certain limiting cases. The more interesting "middle values" (those which are not the limiting cases) have only been recently examined with the aid of high-speed computers. From the results of these studies cited below, one can conclude that the most intense scattered fields are located within a small cone (5°) in the forward direction.

Scattering in a Clean, Dry Atmosphere

The laser beam propagating in a clean atmosphere will be subject to turbulent and molecular scattering and possibly associated molecular absorption. This latter effect must be small if the laser is to be very useful. The most complete measurements of atmospheric transmission have been made using the sun as a

* This quite common assumption is exact for normal, relatively incoherent light. With lasers, where coherence lengths are many meters, coherent scattering might prove important. This is further discussed below.

General method of approach (continued)

source. Figure 1 shows a typical total atmospheric absorption spectrum for vertical transmission to 11 km and 0 km above sea level (1). For horizontal transmission at low altitudes, one must add or subtract appropriate components (e.g., increase the H_2O absorption but remove O_3 absorption.) Figure 2 shows a typical IR horizontal transmittance curve for a 5.5 km path (2). From Figures 1 and 2, one can infer that laser transmission, both vertically and horizontally, will be within the two principal windows--the quasi-visible (0.3 to 2 microns) and the middle infrared (8 to 13 microns)

Atmospheric turbulence will affect the entire band in a very similar manner. The theory of laser beam propagation in the turbulent atmopshere has been thoroughly discussed recently by Davis (3). His results, a development based on the work of Tatarski (4) suggest that the principal laser hazard associated with turbulence is beam steering rather than scattering. For plane waves the turbulent scattering typically (4) leads to intensity fluctuations (scintillation) in the order of:

$$(\log \frac{I}{I_o})^2 = 1.23 \; C_m^2 \; (\frac{2\pi}{\lambda})^{7/6} \; R^{11/6}$$

where I/I_o is the ratio of the maximum intensity to the average intensity, C_m is the structure constant characterizing

General method of approach (continued)

the turbulence (10^{-8} to 10^{-6} $m^{-1/3}$), R is the range in meters and λ the wavelength. Typical curves predicted and measured by Tatarski confirmed in many recent measurements (e.g., Goldstein et. al, (5)) are shown in Figure 3. The eye's aperture is too small to do much averaging so the intensity variations will be as shown in the curve. The immediate conclusion is that beams which are well below damage threshold without turbulent scattering will not normally become dangerous because of scintillation effects over ranges to a few kilometers. Figure 3 shows that for longer ranges, scintillation effects can lead to substantial hazard.

If the beam intensity is above hazard limits, atmospheric beam steering, which causes the entire beam to meander about within a narrow cone, could direct the beam into an unprepared observer's eye. This hazard is confined to very narrow angles (typically several to tens of microradians). For example, Goodwin of Hughes Research Laboratories reports deviations of the order of 50 microradians over a 29 km horizontal path. This would correspond to the center of the beam arriving 1.5 meters from its designated target. For comparison, typical recollimated beam divergences for a gas laser are of the order of tens of microradians. Thus, over the 29 km path, the beam occasionally would meander a full beam width from its mean position. The

General method of approach (continued)

be reasonably constant so the absorption coefficient will be linearly proportional to the partial pressure of CO_2.) The next question to ask is: What happened to the lost energy? If it were strongly absorbed, one would expect it to appear as thermal energy. On the other hand, with a CO_2 partial pressure of 0.152 torr, collisional de-excitation is highly unlikely in an excited lifetime (7) ($\tau \approx 0.3$ milliseconds). Second absorptions are also extremely unlikely. Therefore, it is not unreasonable to assume that the energy is reradiated. It is important to note that the mean time between collisions is $\approx 10^{-10}$ seconds. Thus, the polarization and directionality associated with the absorption of a photon are lost before the photon is reradiated. Each excited atom simply contributes, on the average, a spherical wavelet. Adding these along the entire path emphasizes scattering along the beam axis simply from geometric considerations. However, the $\frac{1}{r^2}$ dependence prevents the development of any substantial scattered field. Accordingly, resonant molecular scattering should not present much of a hazard.

Scattering in Atmospheric Aerosols and Haze

It is obvious from visual observation that fog and snow scatter light in all directions. The details of the process, however, are far from obvious.

General method of approach (continued)

expectation values for r.m.s. angular deviations vary approximately as $R^{0.6}$ so one may extrapolate to other ranges.

Molecular scattering becomes important only when there is a gas species present with an appropriate absorption line. (Nonlinear interactions such as Raman and Brillouin scattering are important only at extremely high intensities more appropriate to laboratory experimentation. They are not considered in this portion of the report.) The most obvious atmospheric gas which will result in this type of interaction is CO_2 where the radiation is from a CO_2 laser. The usual laser transition is between two vibrational levels ($00^{0}1$ to $10^{0}0$) that lie, respectively, 0.303 and 0.17 ev above the ground state. At normal temperatures a significant fraction of the atmospheric CO_2 will be found in the $10^{0}0$ level where it will absorb the laser radiation. Even in uncontaminated air, CO_2 represents 200 ppm of the atmosphere. One can estimate the attenuation coefficient for the CO_2 laser radiation in a clean atmosphere by comparing line strengths and attenuation coefficients with the 4.7 μ CO_2 line. Using the values of Burch et.al. (6) and ignoring the obvious problem of the rotational structure of the absorption line, one obtains a value of $\alpha = 0.05\ km^{-1}$. Thus, in a 20 km horizontal path, the intensity would drop to 1/e of its initial value. (The molecular extinction coefficient will

General method of approach (continued)

Two classes of studies have been carried out which enable one to make reasonably quantitative estimates of the scattering. First, there has been the development of the Mie theory for scattering by a spherical homogeneous particle. The first development in detail was by Stratton and Houghton (8). The result of this work is a rather difficult-to-evaluate integral (or series) which gives the scattered intensity as a function of angles θ and φ for a sphere of normalized radius $x = \frac{2\pi r}{\lambda}$ and complex index of refraction n. Until recently only the limiting cases of $n-1 \ll 1$ (Rayleigh-Gans scattering) and $\sim \ll \lambda$ (Rayleigh scattering) had received detailed analysis. The advent of high speed computers has allowed evaluation of almost any combination of $2\pi\ r/\lambda$ and n. The work of Plass (9) is probably the most extensive to date.

The second development was the measurement of the actual particle diameters of atmospheric contaminants. With solids, this is not too difficult, but with liquids, especially all-important water, it has proved incredibly difficult. After much effort and a great deal of ingenuity, some early data were obtained such as that of Houghton (10) shown in Figure 4a. The early work tended to underestimate the density of the smaller particles. Recent work has shown that the distributions are very strongly

General method of approach (continued)

dependent on both the seeding particles (e.g., sea salt, traces of HNO_3 or H_2SO_4, dust) and on atmospheric conditions. Generally, though, distribution of water droplets in fog has one or several maxima between 1 and 50 microns. (See Figure 4b for typical cloud measurements.)

In nominally clear air, there is reason to suggest that unstable distributions of minute water particles (less than 1 micron) are being generated at turbulent domain interfaces (11).

Particulate haæs are found to consist, in our civilization, primarily of industrial and automotive waste products. Particle sizes are often rather uniform and frequently of the order of 0.1 to 1 micron. Their optical characteristics are quite varied--black or opaque for carbon, high index of refraction, but transparent for many oxides, etc. Van der Hulst (12) gives a detailed discussion of their scattering properties (data to 1957). The work of Plass (9) gives more recent calculations.

With details of the scattering process and the scattering ensemble at hand, one would like to develop graphs of the normalized scattered intensity versus angle and range to determine the appropriate danger zones. This process is quite possible for any given set of boundary conditions. First, some general comments are in order.

General method of approach (continued)

The scattering cross-section, σ_s, of a particle is the ratio of the total scattered power to the incident irradiance. For small particles ($\frac{2\pi a}{\lambda} \ll 1$) the scattering cross-section is much less than the geometric cross-section, σ_g. As the radius increases, the scattering cross-section increases rather rapidly (as λ^4). However, its exact dependence is a function of the complex index of refraction $n = n_1 - jn_2$. From Babinet's principle, one can state that the ratio $Q_s = \frac{\sigma_s}{\sigma_g}$ approaches 2 for a transparent (or white) particle and 1 for a black particle. For an opaque particle of albedo > 0, Q_s approaches a value between 1 and 2. Figure 5a shows Q_s for the important case of $n_1 = 1.33$ (water). For water illuminated at 0.5 microns, $n_2 = 0$. On the other hand, water at 10 microns has an imaginary component of its index of refraction of 0.07. Since $n_2 > 0$ implies absorption as well as scattering, it is appropriate to define σ_a as the ratio of the absorbed power to the incident irradiance and $Q_a = \frac{\sigma_a}{\sigma_g}$. The total attenuation (or extinction) cross-section is then $\sigma_{att} = \sigma_a + \sigma_s$ and $Q_{att} = \frac{\sigma_{att}}{\sigma_g}$. Figures 5b and 5c show Q_a and Q_{att} for several values of n_2. It is interesting to note that Q_a is at the highest value for just those conditions that describe CO_2 laser radiation propagating through a typical atmospheric water aerosol: $n_2 = 0.1$ with x ranging from 0.6 to 38.

General methods of approach (continued)

On the other hand, Q_s is much more significant for visible radiation since x ranges from 13 to 755 for visible light.

Two other pieces of data are necessary to estimate the danger zone. First, Figure 6 gives the scattering coefficient, $i(\theta)$ as a function of angle for three values of x for $n_1 = 1.33$ and $n_2 = 0$ and 0.1. It will be noted that: small x leads to almost equal forward and backward scattering, but as soon as the particle circumference is much larger than the wavelength, the scattering is almost entirely in the forward direction. From the graph for x = 40 (e.g., 4.5 μ, particles illuminated by ruby laser light) it can be seen that the intensity has fallen by two orders of magnitude in 10 degrees. Accordingly, one can state that the principal danger zone for droplet scattering lies within a narrow cone about the direction of propagation.

To obtain an order of magnitude measure of the hazards involved, consider what would happen with a ruby laser emitting a beam of 1,000 watts/cm^2 at 0.694 microns. Figure 7 shows how $i(0)$ depends on x. To obtain the intensity in watts/steradian for an illuminating intensity of 1 watt/cm^2, one multiplies $i(0)$ by $\lambda^2/4\pi^2$ with λ in centimeters. For our example of 1 kw/cm^2 and λ = 0.694 microns, a single 20 micron particle (x = 182) yields 34.8 watts/steradian in the forward

General methods of approach (continued)

direction; while at 10.6 microns (x = 12) it yields only 0.112 watts/steradiam.

For purposes of estimating the hazard, let the particle radii be 20 microns and its density equivalent to the water vapor in the air at 40% relative humidity at 12°C. That is $\frac{1}{3} \times 10^{-2}$ gm of water/gm of air. Converting this to droplets and assuming the beam is 1 cm^2 in cross-section, one obtains 3×10^3 particles per meter of path. To obtain an intensity estimate, one must integrate the scattered light over the whole path with its usual $1/R^2$ dependence. This intensity illuminates the whole eye. To obtain the intensity at the retina, one must find the contribution from the volume that contributes to the most intense diffraction limited spot (roughly 16 μ diameter). The calculation is simple as long as one is far enough away from the beam so that the eye subtends a very small solid angle from the particle (with, say, 5° off-axis limit, this restriction will always be met.)

The whole eye is subjected to an intensity $I_e = \frac{0.9}{d}$ watts/cm^2 where d is the lateral separation between the observer and the beam in meters. Visible light will be focused and thus intensified. If one assumes that light from the nearest just resolvable volume produces the brightest spot, one has $I_{spot} = I_e \left(\frac{\Delta\theta}{\theta}\right) \times \frac{\text{area of pupil}}{\text{area of spot}} \approx 200\, I_e \cdot \left(\frac{\Delta\theta}{\theta}\right)$ is the

General methods of approach (continued)

percentage of the incident intensity that is focused into the diffraction limited spot and the area ratio gives the optical gain of the eye.)

A plot of I_e versus d is given as Figure 8. This plot, of course, corresponds to the given set of conditions which were rather artificial. However, several very interesting factors should be discussed. First, the density of particles under discussion corresponds to a moderate fog with an attenuation factor of 20 nepers/km. For comparison, clean air at sea level has an α of 0.02/km and moderate haze would be about 3/km. Fogs run as high as 200/km.

Second, the result is independent of the beam cross-section (within the limits of subtending no more than 5° at the eye). This follows from the fact that doubling the cross-section halves the power density but doubles the number of scatterers.

Third, the result is linearly dependent on the radiated power. Finally, the result of increasing the wavelength is to decrease the scattered intensity (in accordance with Figure 7). Note also that it is Q_s that is small for the 10μ radiation. Q_a and thus Q_{att} is large so the 20μ particles will attenuate 10μ radiation much more strongly than they will scatter it. For this example of a 20μ monodispersion, the difference is a

General methods of approach (continued)

factor of 190 between the ruby laser and the CO_2 laser. Finally, it should be noted that the assumed power - 1 kw - is not unrealistic for high power CW transmission in the present or near future. However, for pulsed operation peak powers in excess of 10^{+7} watts are routinely generated in radar applications. To obtain a better measure of hazard in that case, a value of 10 joules transmitted per pulse (or per eye integration time) is a reasonable boundary. Assuming a pulse of 10^{-6} seconds gives a power of 10^{+7} watts. Thus, $I_e = \frac{9}{d}$ kw/cm^2 and $I_{spot} \approx \frac{1.8}{d}$ megawatts/cm^2. Integrating for the pulse duration gives energy densities of $U_e = \frac{.009}{d}$ joules/cm^2 and $U_{spot} = \frac{.18}{d}$ joules/cm^2, respectively, (where d is in meters).

It should be reiterated that the numbers given in the above example are very rough estimates based on a rather artificial example. These numbers indicate that a substantial hazard exists for an observer standing within several meters of a powerful laser radar beam at visible wavelengths in a moderate fog. If the weather was described as a "light fog" ($\alpha = 2$ km^{-1}), the danger zone would be reduced to well within a meter or two of the beam. It is more difficult to draw so sharp a boundary for the CW case. If one assumes the eye would blink in a time of the order of 10^{-2} seconds, the critical distance for the 1 kw

General methods of approach (continued)

laser at the same visible wavelength is about the same as for the 10 joule pulse. On the other hand, to achieve a particular safety limit--say an intensity of less than 10 watts/cm^2 on the retina spot--one should not come within about 10 meters of the beam, as an initial very rough estimate. Such a conclusion has obvious bearing on the appropriate ground clearance for high power laser transmitters. Further study of this problem is required.

The Problem of Snow

Scattering from snow flakes is a subject almost more appropriate to "semi-diffuse reflectance" than atmospheric scattering. A typical snowflake would intercept a substantial portion of a 1 cm^2 beam. A priori, one would guess that randomly tumbling snow flakes would attenuate a laser beam's energy very rapidly. One might also expect to find large scintillations in the intensity observed at any angle. If the beam were rapidly attenuated, the random angle scattering near the transmitter would pose the principal threat.

Data on beam attenuation and average beam width for a laser beam propagating in snowy weather have been published by Hogg (13). His data are reproduced in Figure 9. It is important to note that the curves have been normalized to 0 db

General methods of approach (continued)

at the center of the beam. The attenuation at the center is given on the individual curves. Hogg's data did not include meterological data so that it is difficult to say more than "heavy snow gives high attenuation."

Figure 10, derived from Hogg's data, shows that beam broadening increases much more slowly than beam attenuation. Two conclusions may be drawn from Figure 10: First, forward scattering is not dominant in snow. Second, there is no new hazard for near 0° observers as there is for the case of light fog or rain.

On the question of large scintillation at large angles, no data are available. However, since snow does not absorb visible light, and the beam attenuation is high, it follows that scattering is the principal effect. For 10μ radiation, the albedo may be substantially less than 1. From the shape of a snow flake, it would seem probable that at each instant of time the beam would be strongly deflected in some particular direction or directions with little or no fields at other angles. Since such behavior would constitute a substantial and rather unpredictable hazard, an experimental investigation of large angle, visible and infrared scattering in snow should be undertaken.

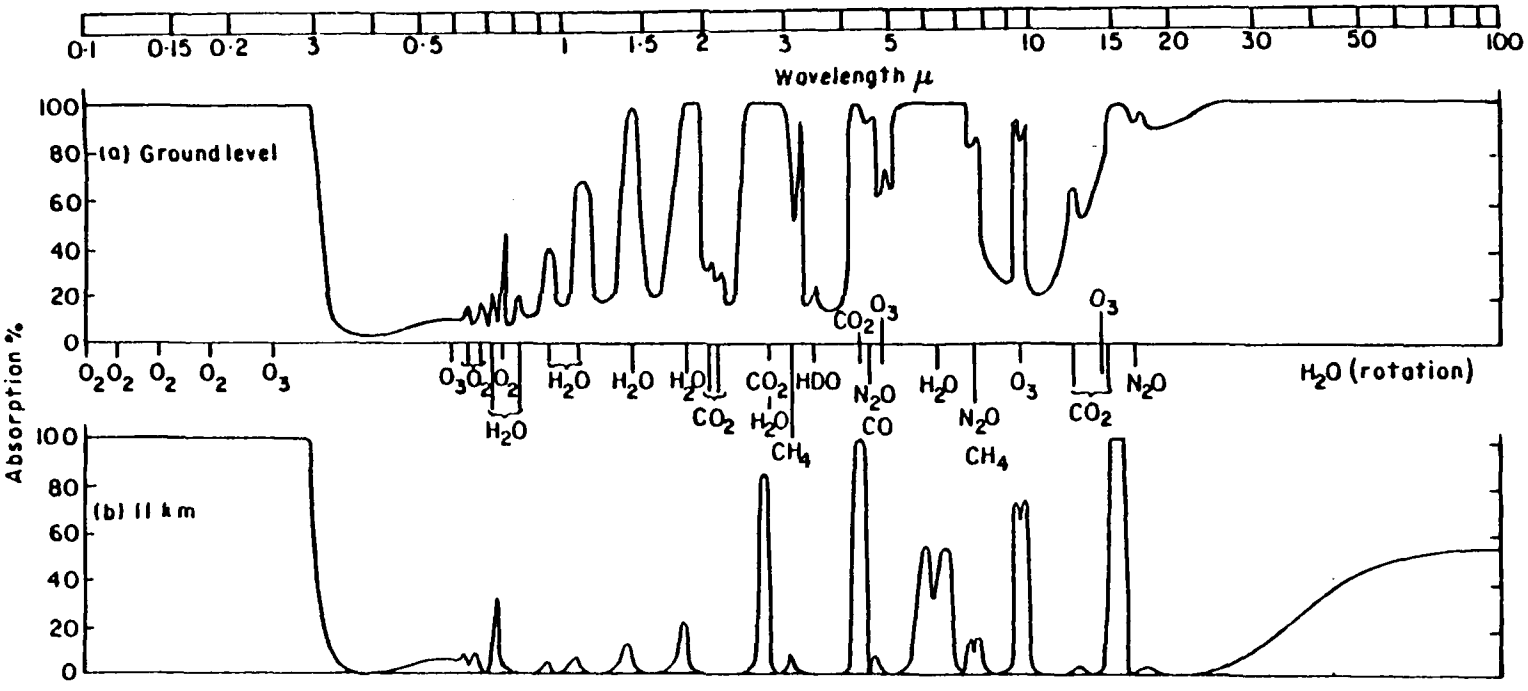

FIGURE 1 Atmospheric absorptions.
(a) Atmospheric gaseous absorption spectrum for a solar beam reaching ground level. (b) The same for a beam reaching the temperature tropopause.

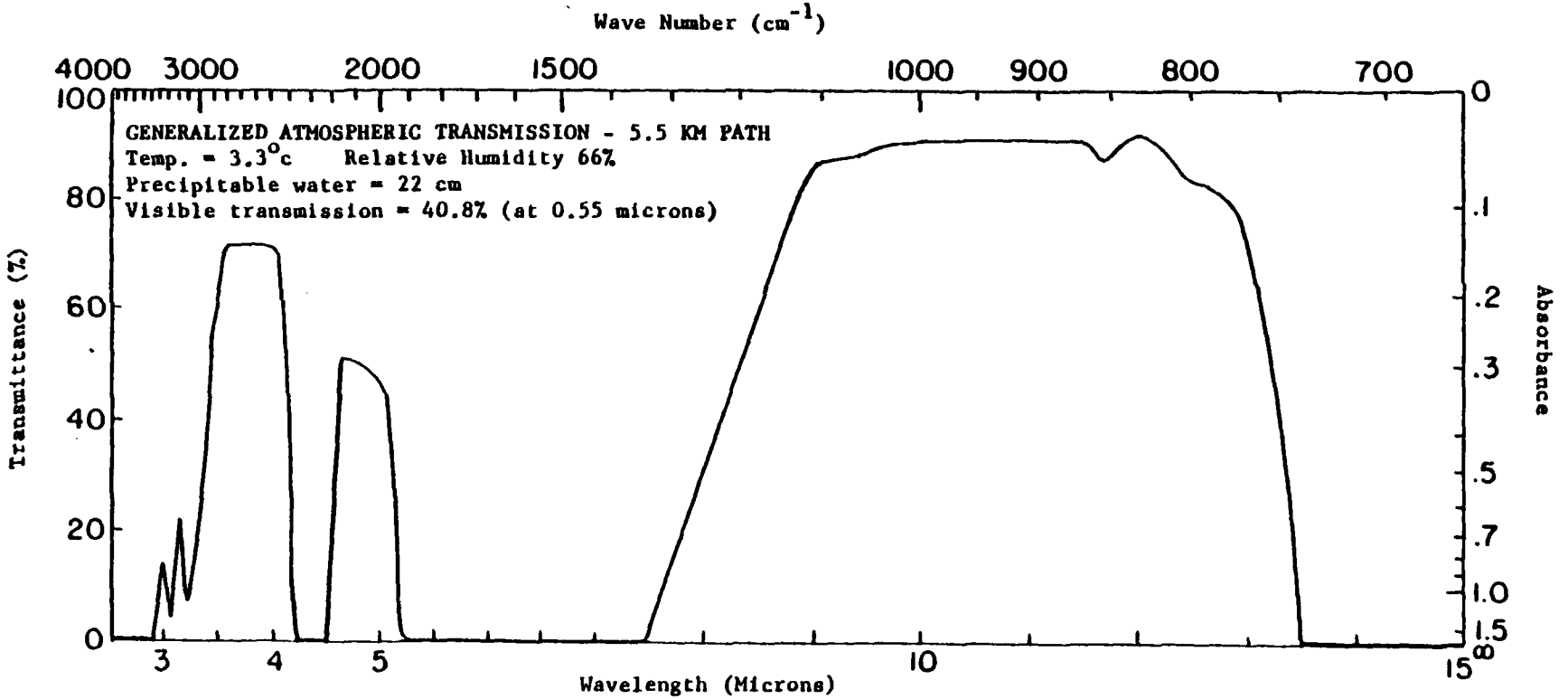

Figure 2

Typical atmospheric transmission

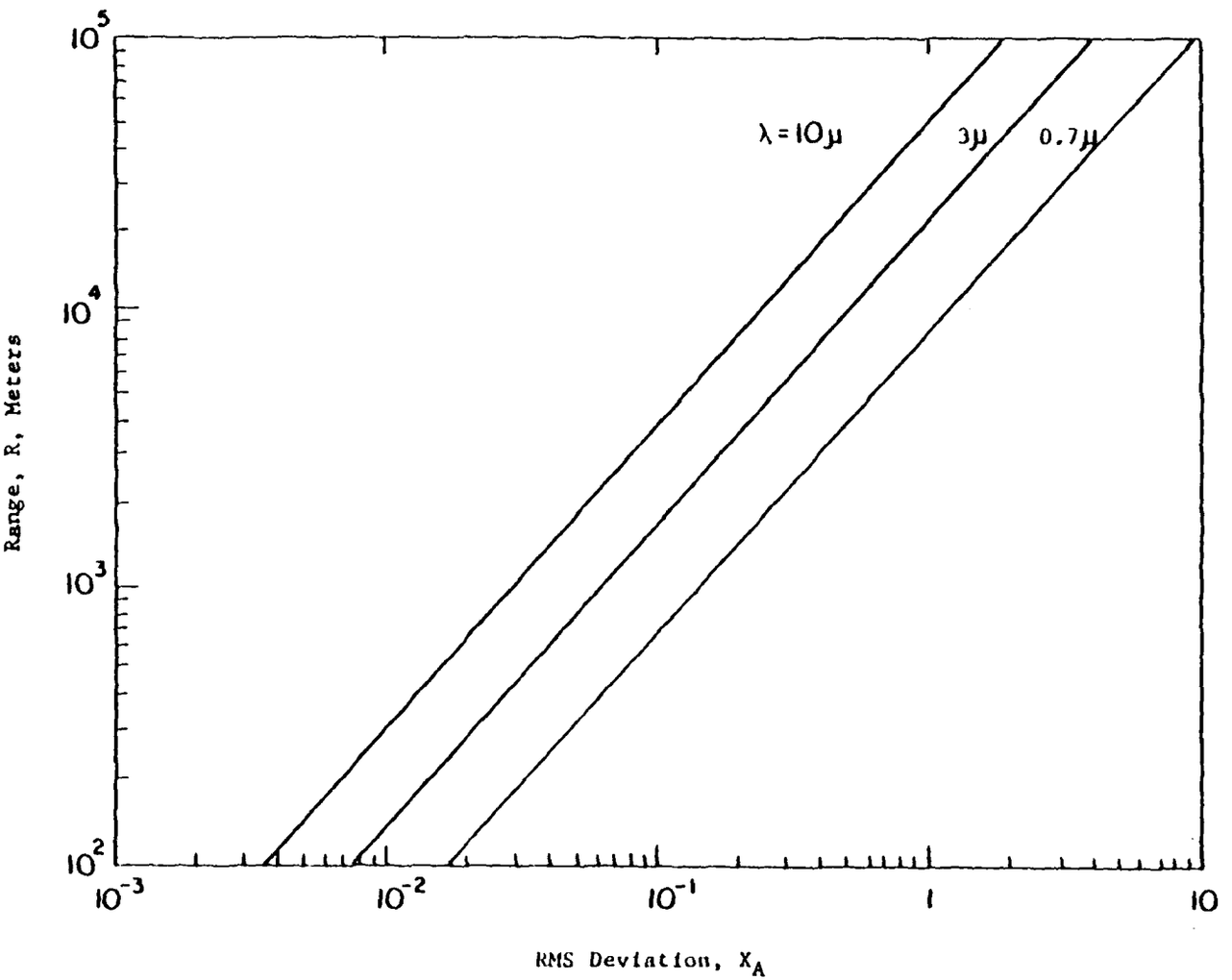

Figure 3

Range, R, vs standard deviation in logarithmic level of amplitude, X_A, for intermediate turbulence.

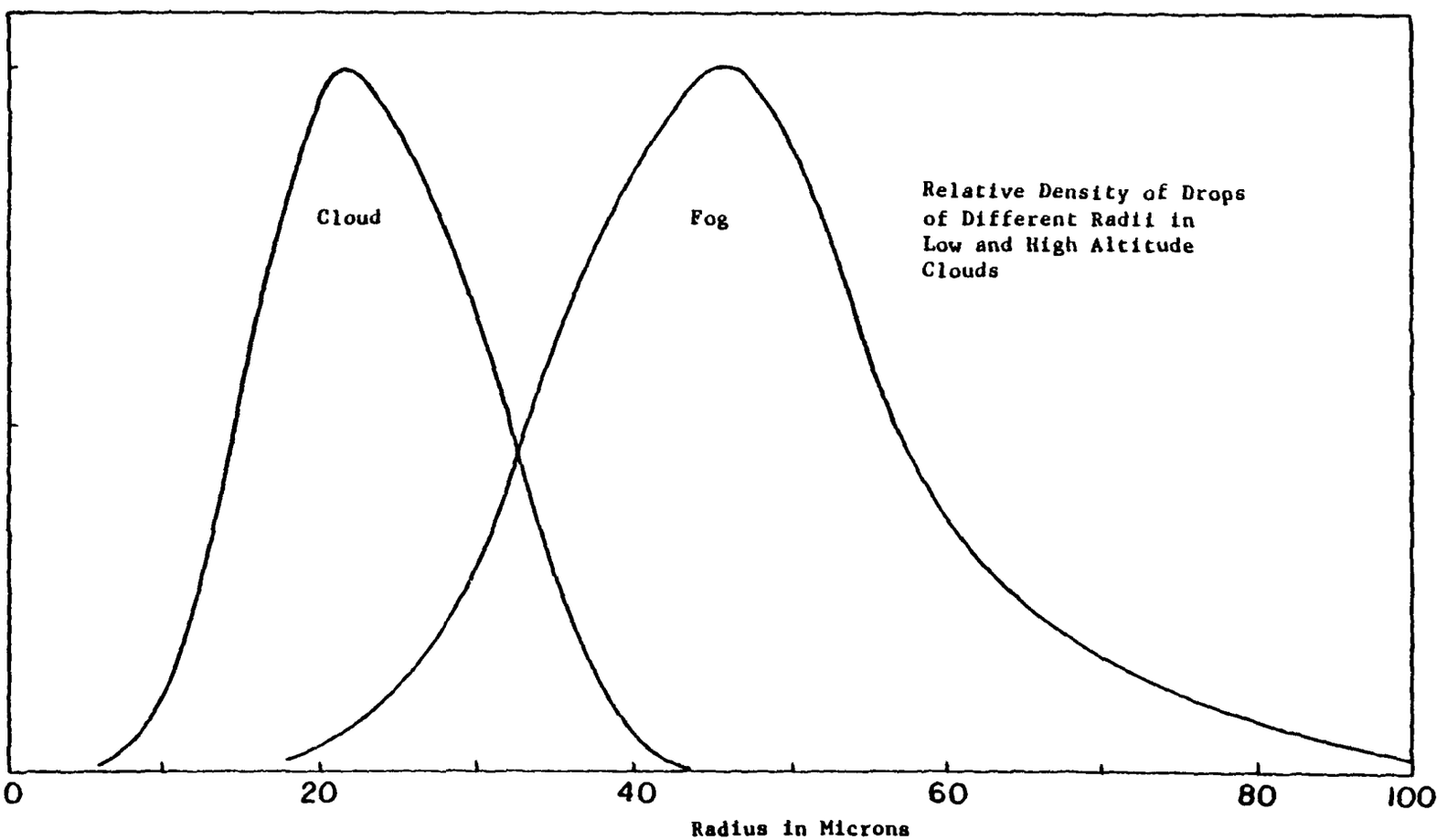

Figure 4a

Cloud drop-size spectra .

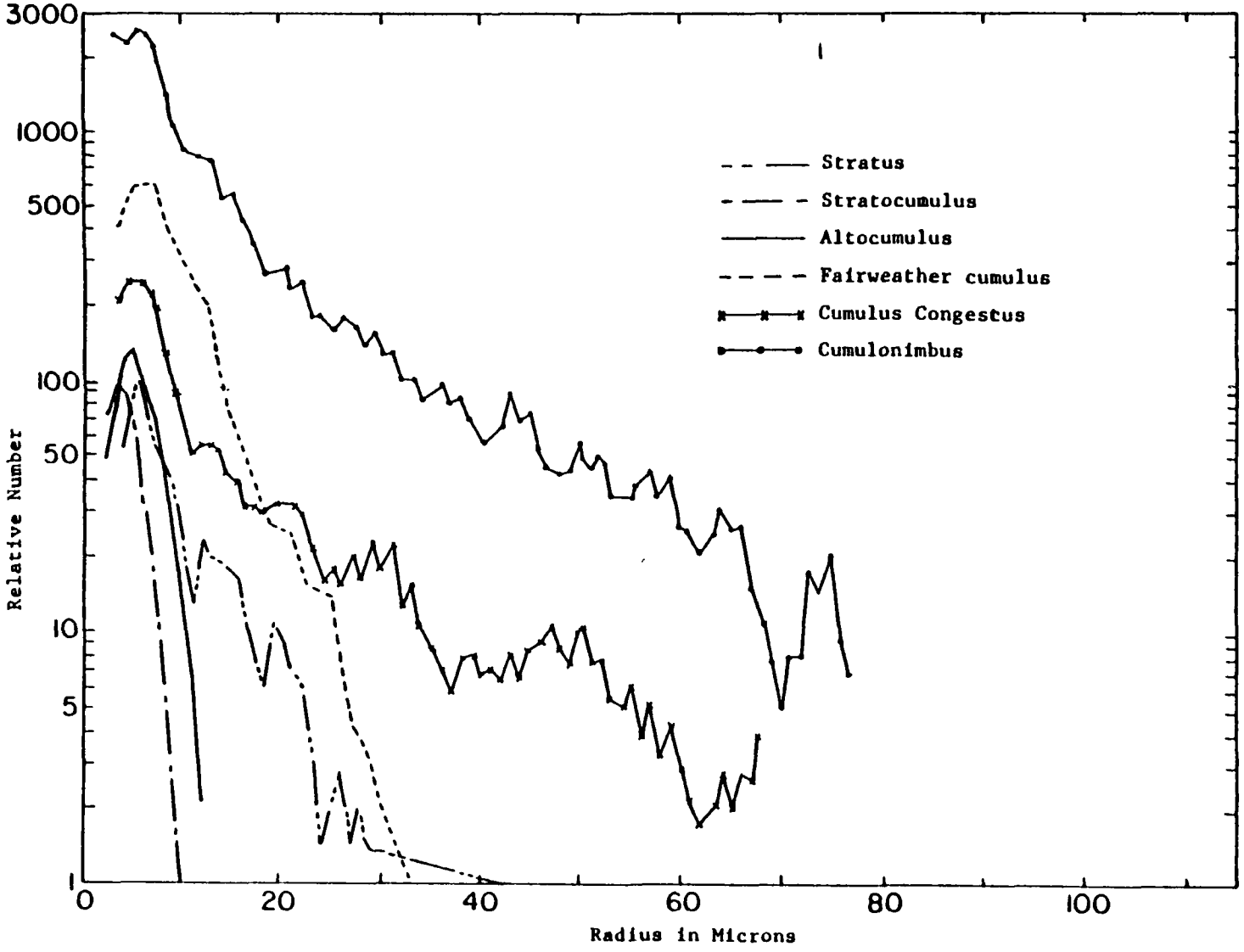

Figure 4b

Cloud drop-size spectra

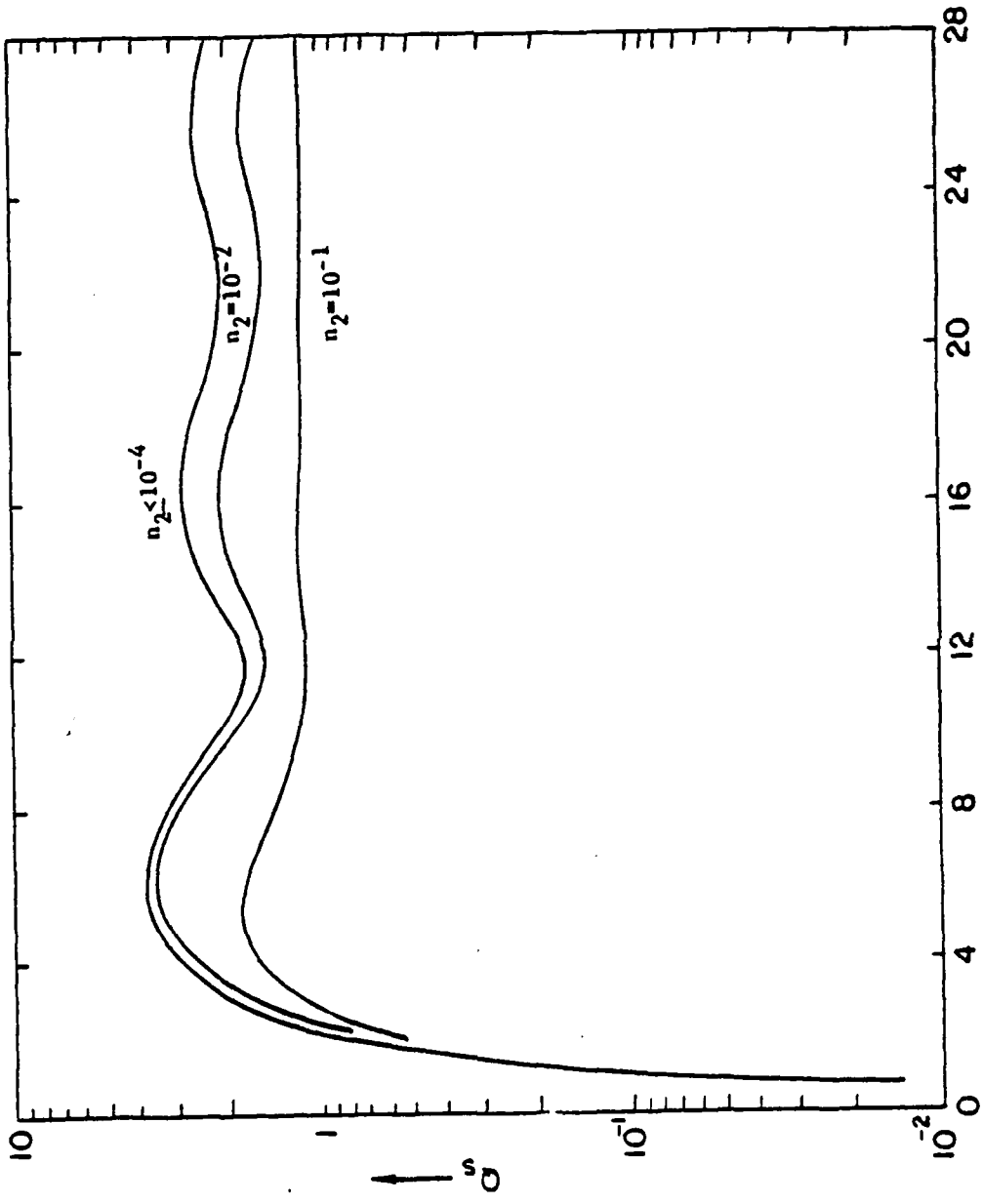

Figure 5a

Efficiency factor for scattering as a function of $x = 2\pi a/\lambda$ for $n_1 = 1.33$

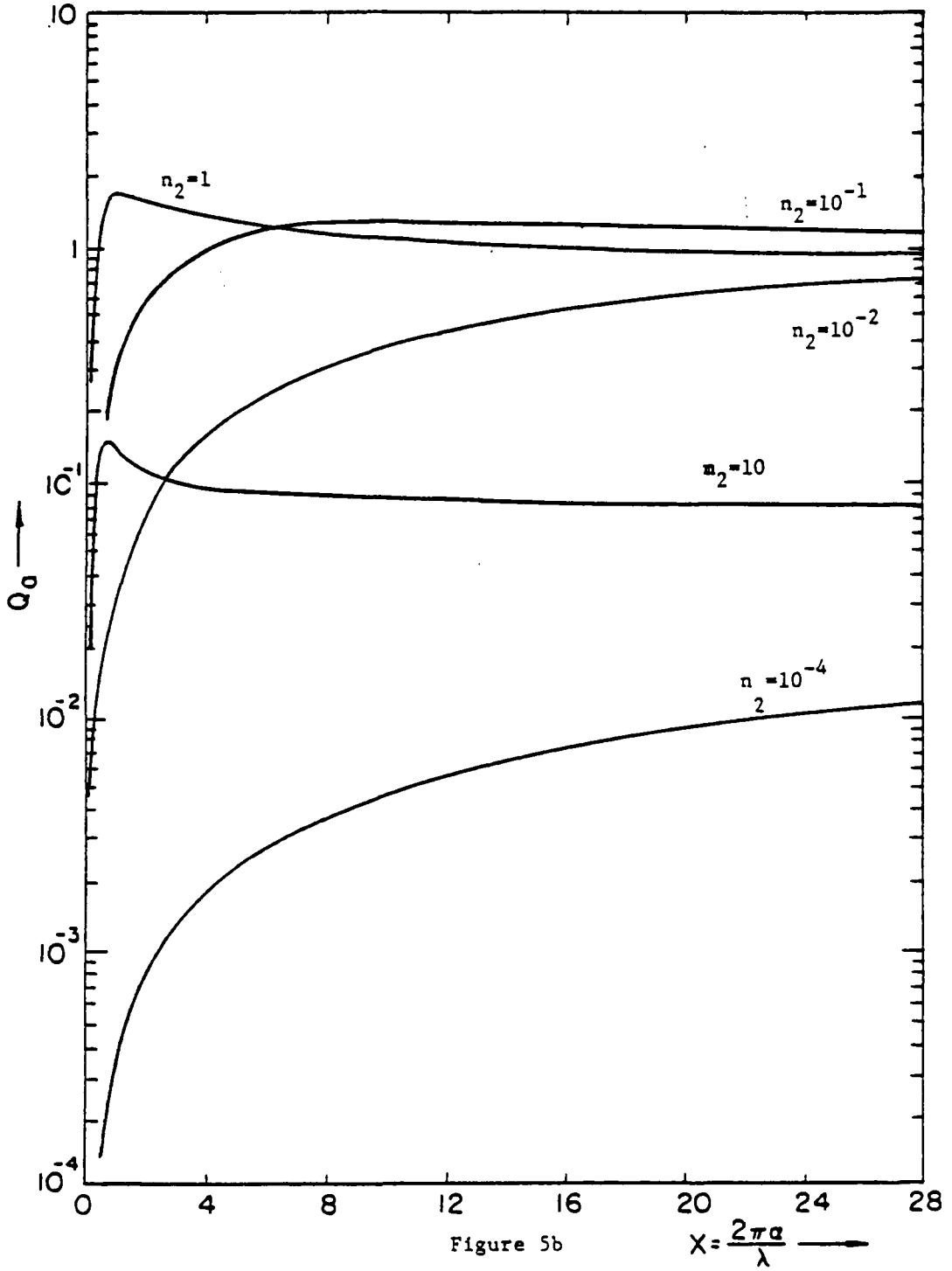

Figure 5b

Efficiency factor for absorption as a function of $x = 2\pi a/\lambda$ for $n_1 = 1.33$.

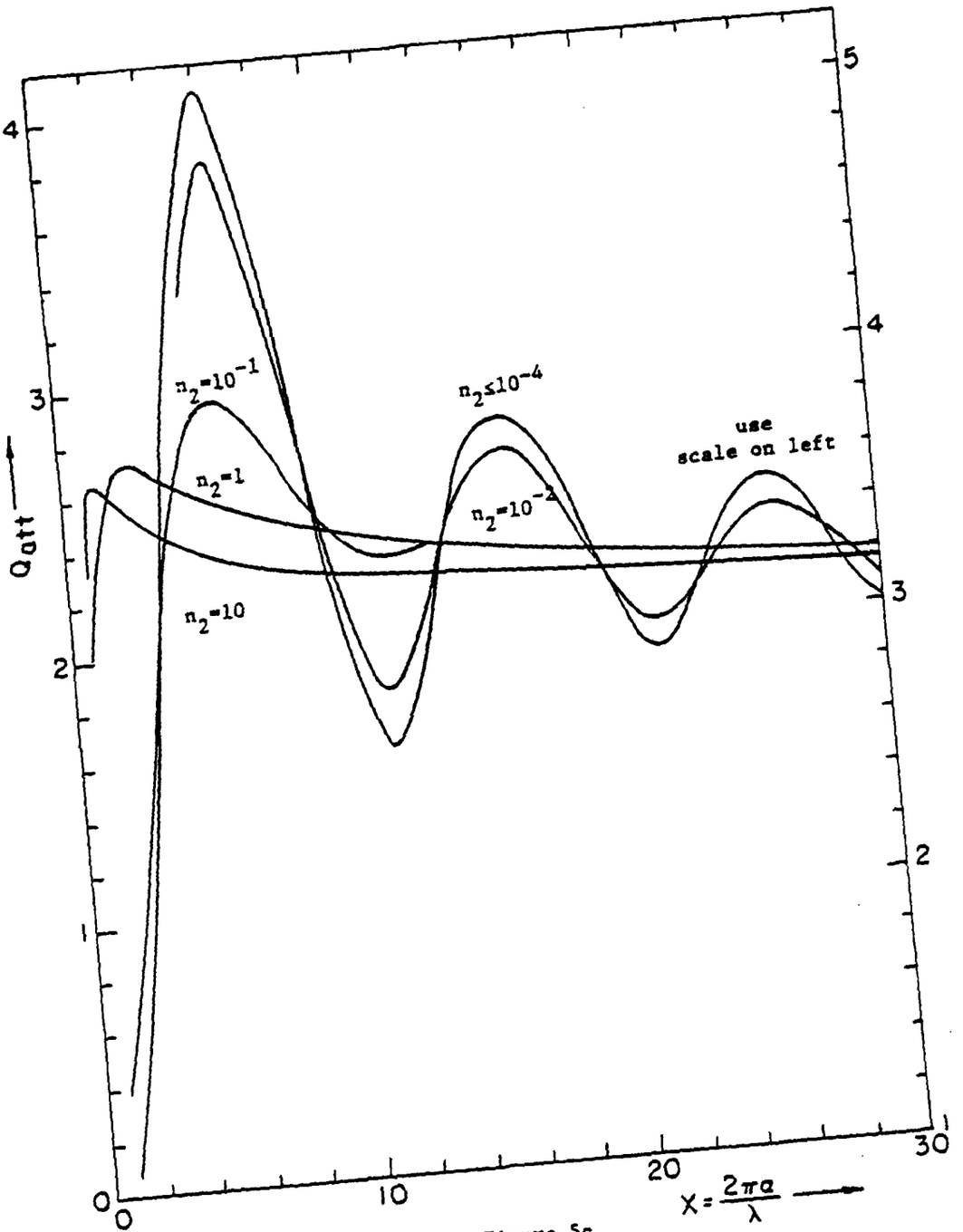

Figure 5c

Efficiency factor for extinction as a function of $x = 2\pi a/\lambda$ for $n_1 = 1.33$

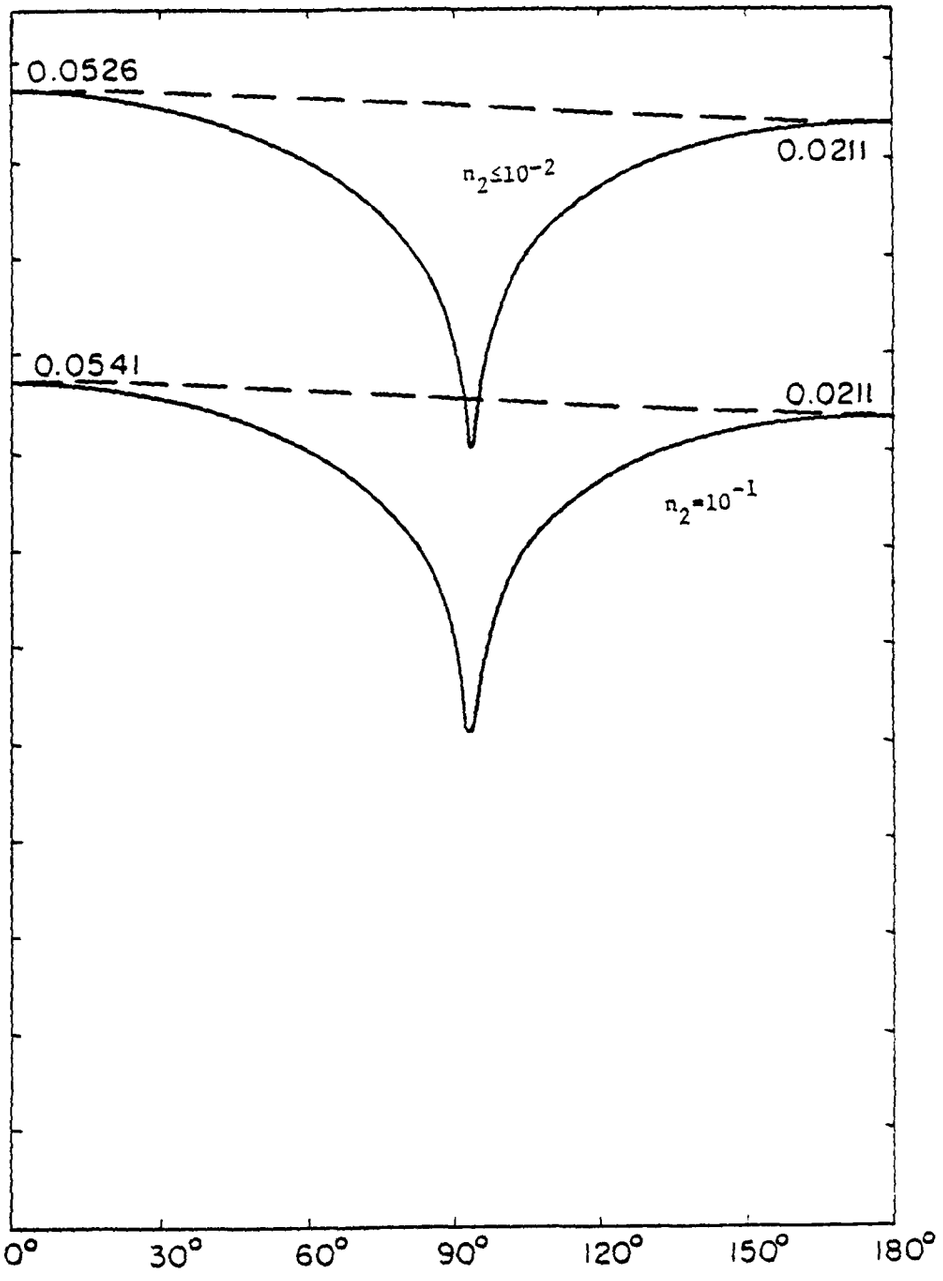

Figure 6a

Scattered intensity as a function of scattering angle for $n_1 = 1.33$ and $x = 2\pi a/\lambda = 1$.

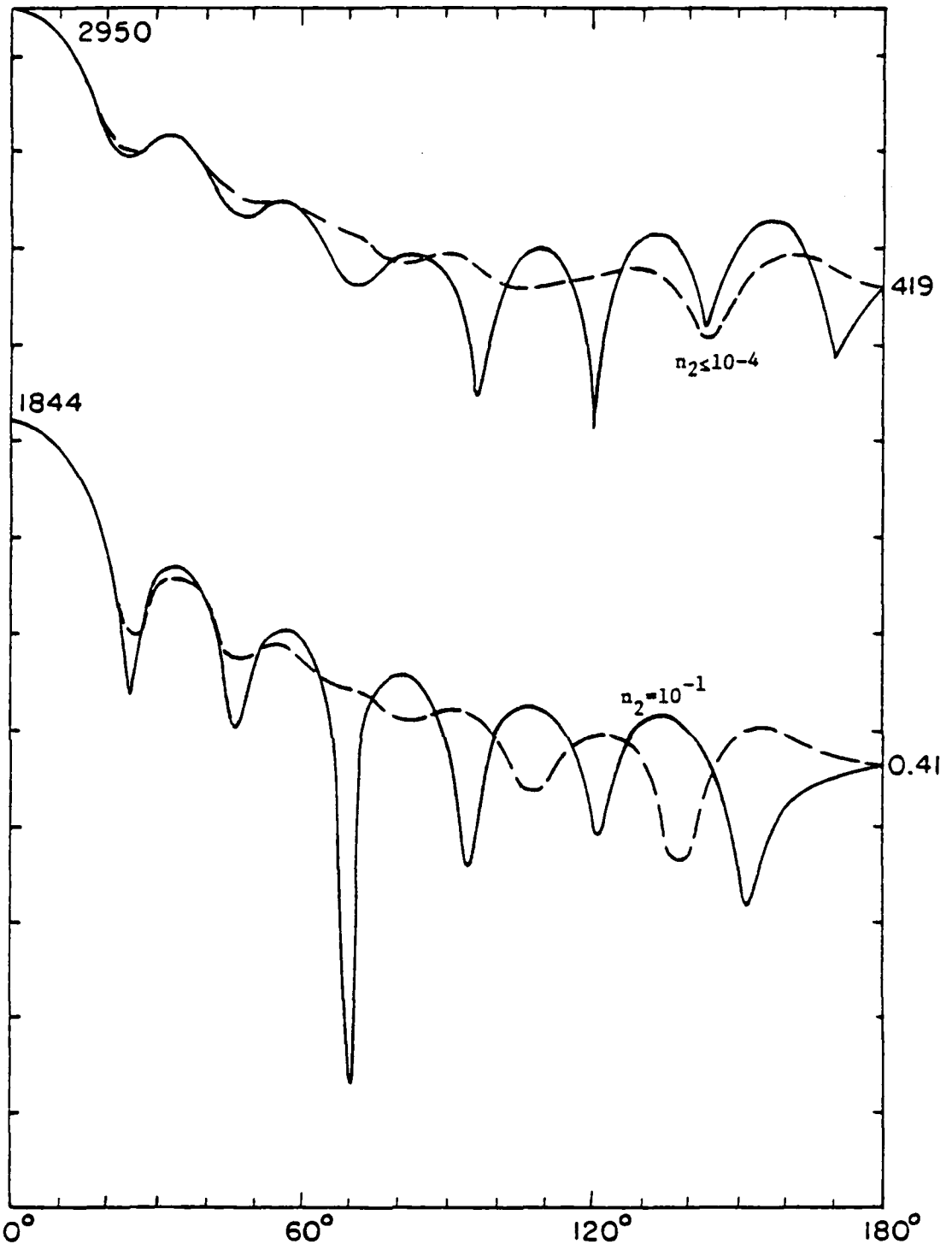

Figure 6b

Scattered intensity as a function of scattering angle for $n_1 = 1.33$ and $x = 2\pi a/\lambda = 8$.

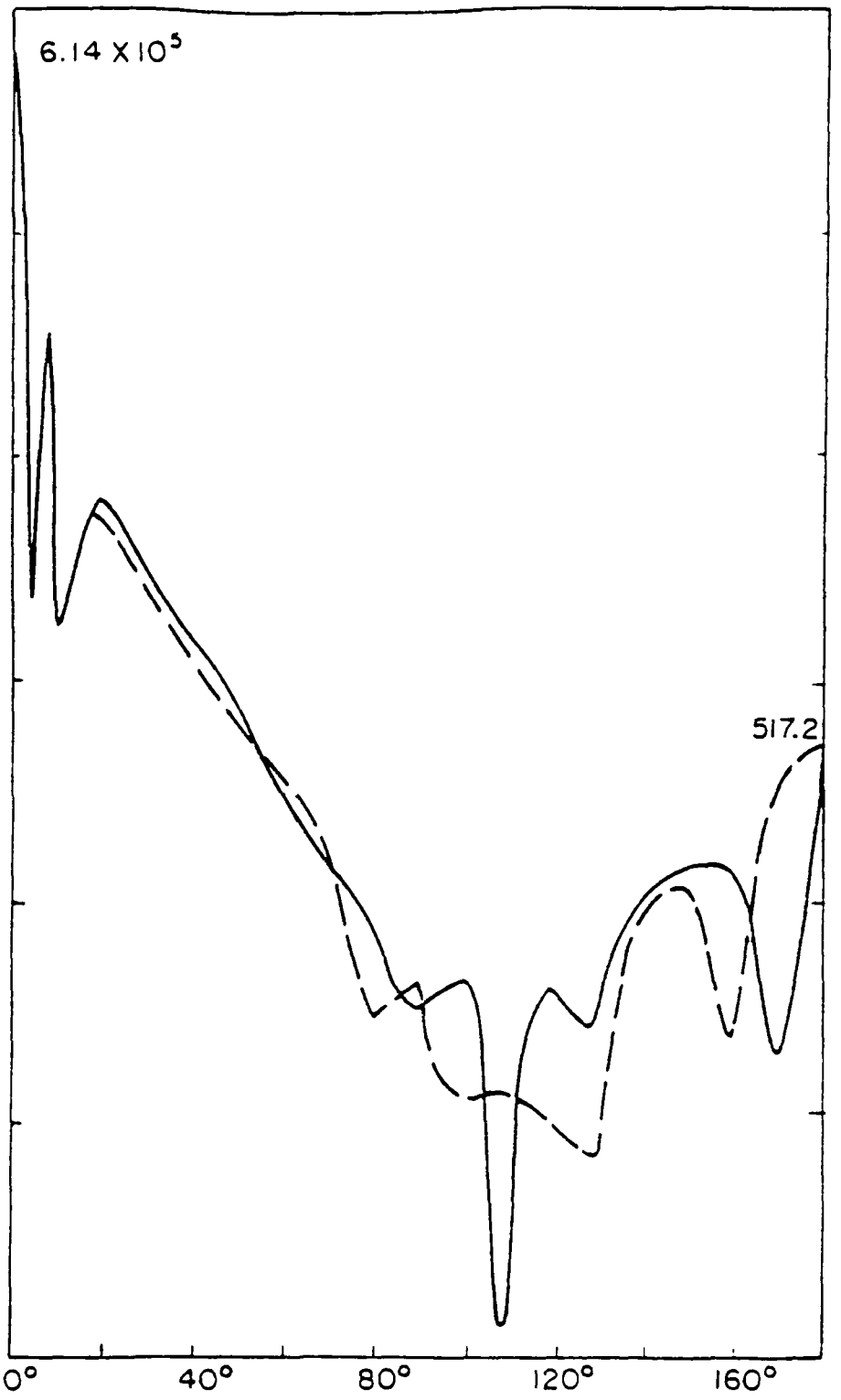

Figure 6c
Angular intensity of light scattered by a spherical particle for m = 1.33 and α = 40.

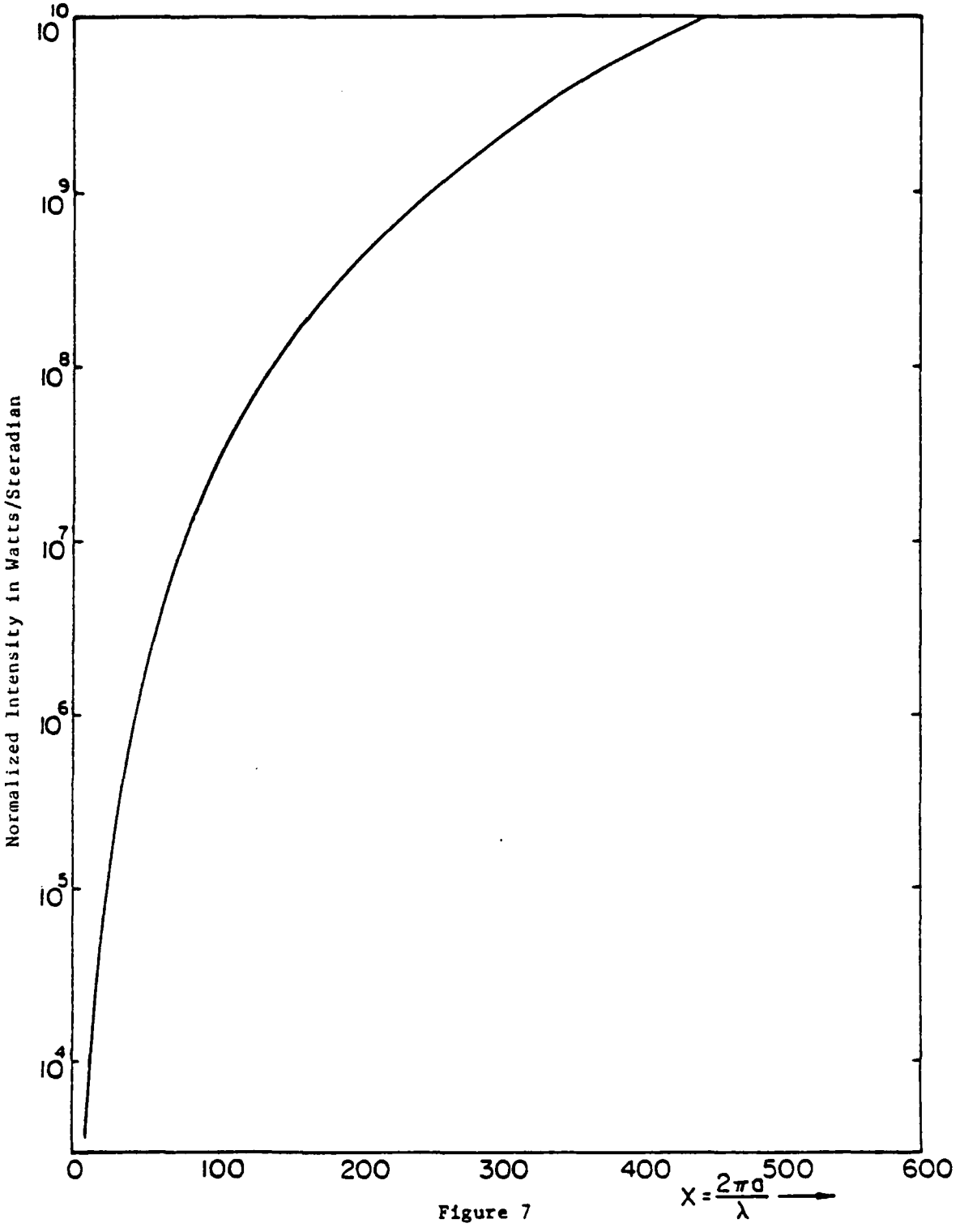

Figure 7

Normalized Scattered Intensity in the Forward Direction vs $x = \frac{2\pi a}{\lambda}$

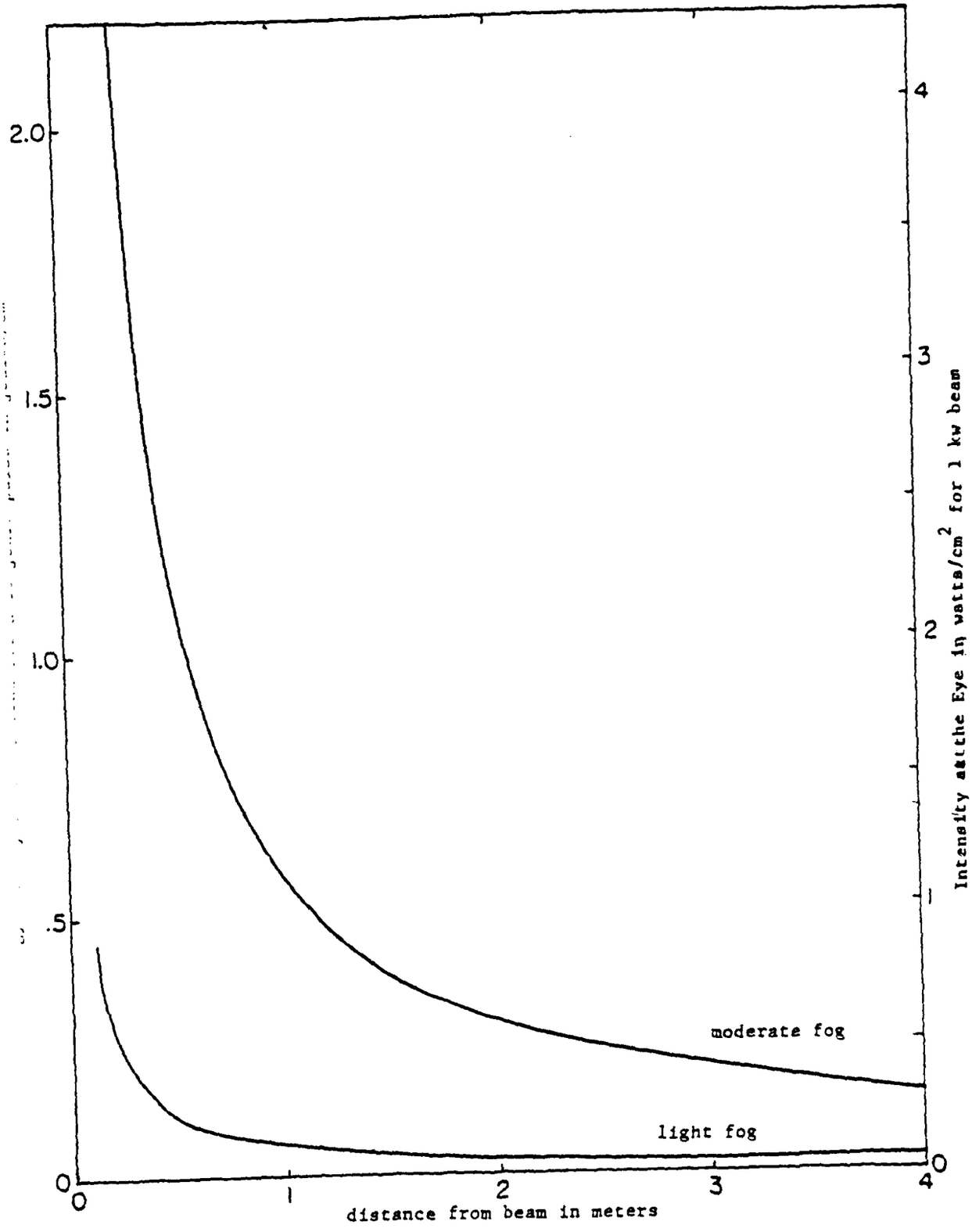
2.0
1.5
1.0
.5
0
0
1
2
3
4
4
3
2
1
0
moderate fog
light fog
distance from beam in meters
Intensity at the Eye in watts/cm^2 for 1 kw beam

Figure 8

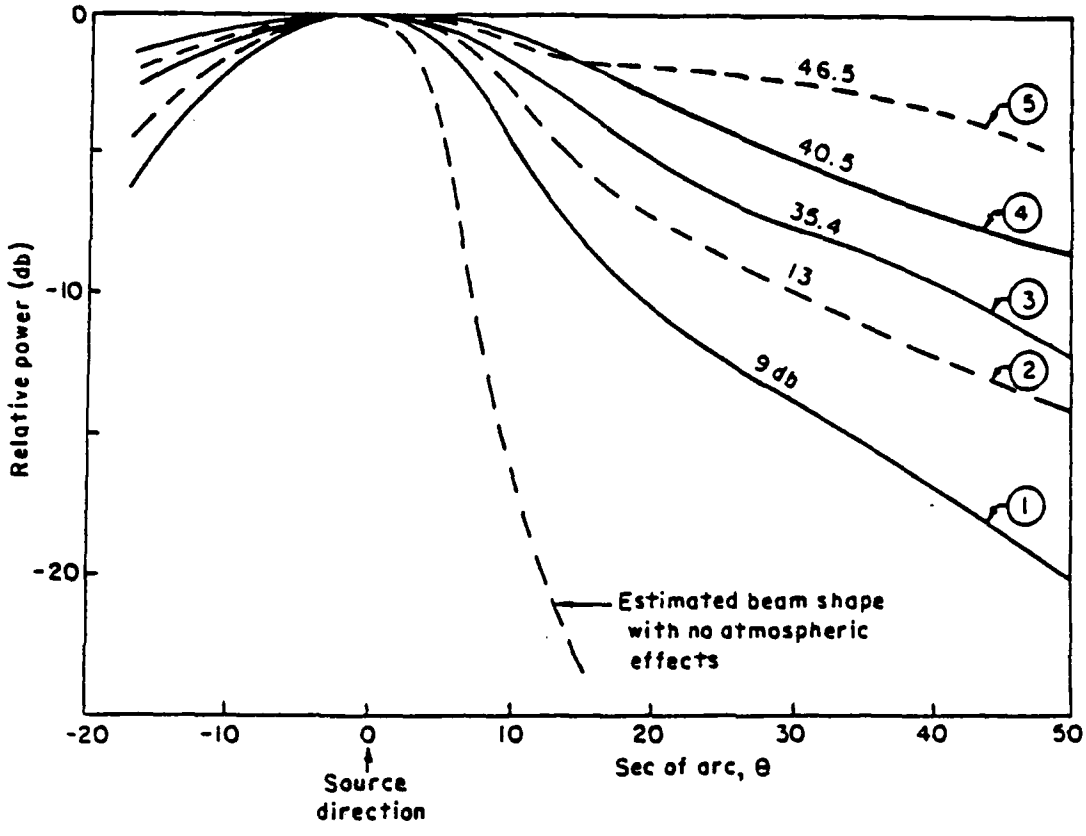

FIGURE 9 Beam broadening due to snow: $\lambda = 0.63$; path length, 2·6 km. Data of 2/10 and 2/11/64. Excess attenuations in curves 1-5 are 3·5, 5·0, 13·6, 15·6 and 17·9 db/km respectively, corresponding to increasingly heavy snowfalls. The attenuations are measured at dead reckoning, that is, with the receiving aperture on the maximum of the beam pattern. ($\theta=0$)

(by D.C. Hogg)

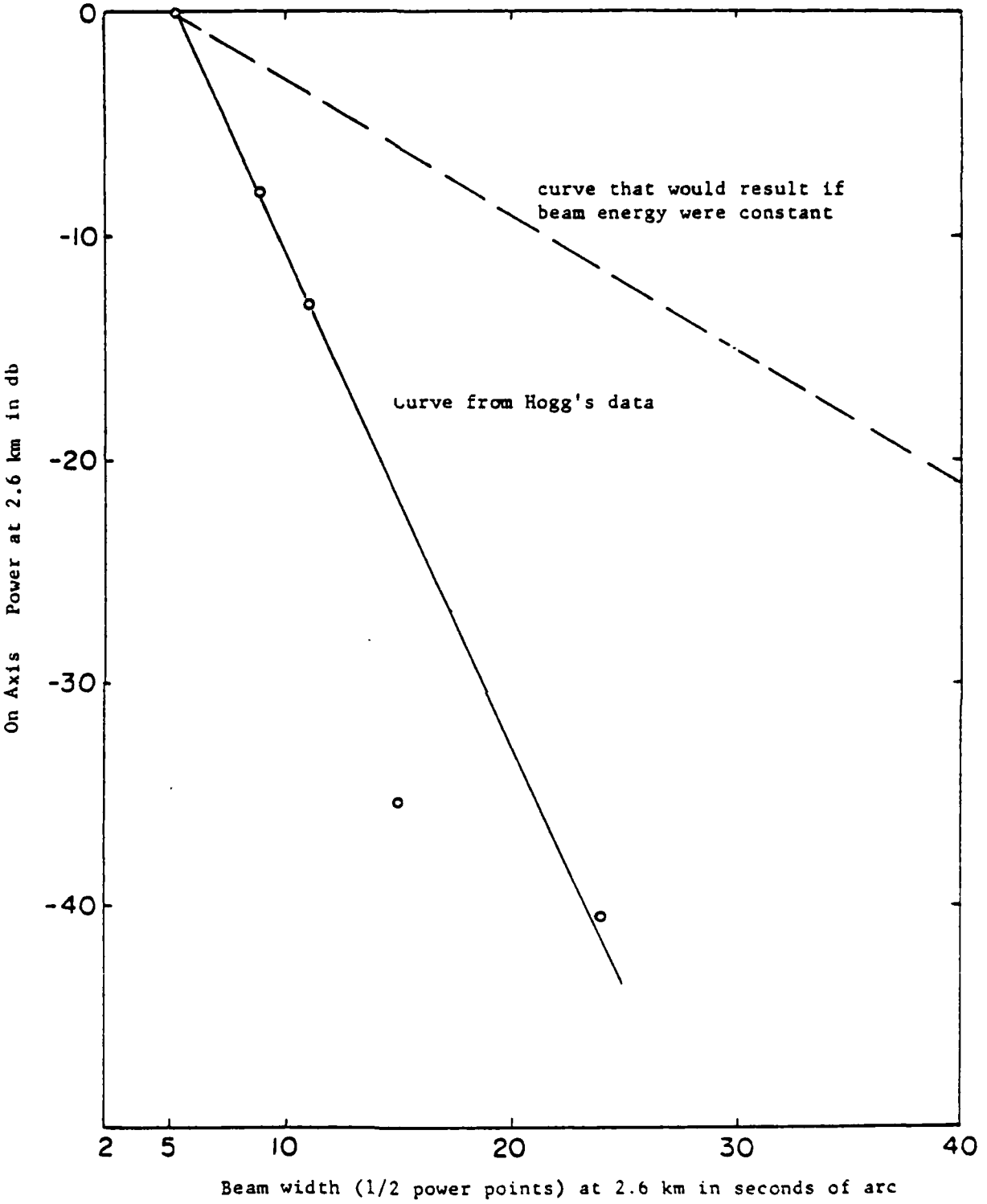
0
-10
-20
-30
-40
On Axis Power at 2.6 km in db
curve that would result if
beam energy were constant
Curve from Hogg's data
2
5
10
20
30
40
Beam width (1/2 power points) at 2.6 km in seconds of arc

Figure 10.

BIBLIOGRAPHY

1. Goody, R. M., Atmospheric Radiation, Oxford, Clarendon Press, 1964.

2. Taylor, J. H., and Yates, H. W., JOSA 47, 223 (1957).

3. Davis, J. I., Appl. Opt. 5, #1, 139 (1966).

4. Tatarski, V. I., Wave Propagation in a Turbulent Medium, McGraw-Hill , 1961.

5. Goldstein, et. al., Proceedings, IEEE, 53, No. 9, 1172 (1965).

6. Burch, D. E., et. al., Appl. Opt. 1, #6 (1962)
   Also, Curcio, J. A. and Estes Buttrey, D. V., Appl. Opt. 5, #2, 231 (1966)

7. Flynn, G. W., et. al., App. Phys. Let. 8, No. 3, 63 (1966)

8. Stratton, J. A., and Houghton, H. G., Phys. Rev. 38, 159 (1931) and
   Stratton, J. A., Electromagnetic Theory, McGraw-Hill, 1941

9. Plass, Gilbert N., Appl. Opt., 5, #2, 279 (1966) and
   Appl. Opt. , 5, #1, 149 (1966)

10. Houghton, H. G., J. of Aer. Sci., 6, 103-107

11. Carlson, H. R., Appl. Opt., 4, #9, 1089 (1965)

12. Van der Hulst, H. C., Light Scattering by Small Particles, John Wiley, 1957.

13. Hogg, D. C., Nature, 203, 396, (1964)

PUBLICATIONS

A. Laser-Related

1. Fine, S., Klein, E., Scott, R.E., Aaronson, C. and Donoghue, J. "Biological Effects of Laser Radiation," Second Boston Laser Conference, August 1, 1963.

2. Fine, S., Maiman, T.H., Klein, W. and Scott, R.E. "Biological Effects of High Peak Power Radiation," Life Sciences, 3:309-322, March, 1964.

3. Fine, S. and Klein, E. "Effects of Pulsed Laser Irradiation of the Forehead in Mice," Life Sciences, 3:199-207, March, 1964.

4. Fine, S., Klein, E. and Scott, R.E. "Studies on Interaction of Laser Radiation with Biological Systems, " IEEE Spectrum, March, 1964.

5. Fine, S., Klein, E., Derr, V.E. and Nowak, W.B. "Hazards and Biological Effects of Laser Radiation," Proceedings of the Martin Interdivisional Solid State Symposium, March 1964.

6. Edlow, J., Farber, S., Fine, S. and Klein, E. "Prenatal and Neonatal Effects of Laser Radiation," Biological Abstracts of Boston Laser Conference, 1964.

7. Klein, E., Fine, S., Cohen, E., Ambrus, J., Neter, E., Lyman, R. and Scott, R.E. "Effects of Laser Radiation on Biological Systems," American College of Physicians (Atlantic City, N.J.), April 10, 1964.

8. Klein, E. and Fine, S. "Effects of Laser Radiation on Animal Tissues," presented at Conference on Lasers, New York Academy of Sciences, May 4, 1964.

9. Klein, E., Fine, S. and Laor, Y. "Modification of Effects of Laser Radiation by Light Absorbing Chemicals," Biological Abstracts of Boston Laser Conference, August, 1964.

10. Klein, E., Fine, S., Scott, R.E. and Farber, S. "Observations of Laser Irradiation of Experimental Tumors," Proceedings, American Association for Cancer Research, 1964.

11. Derr, V., Klein, E. and Fine, S. "Electron Spin Resonance Tests of Laser Irradiated Biological Systems," Applied Optics, 3:786, 1964.

12. Fine, S., Klein, E., Aaronson, C., Hardway, G., King, W. and Scott, R.E. "Closed Circuit Television in Laser Investigations," Journal of Invest. Derm., 4:289-91, 1964.

13. Klein, E., Fine, S., Laor, Y., Litwin, M., Donoghue, J. and Englander, L. "Laser Irradiation of the Skin," Journal of Invest. Derm., 43:565, 1964.

14. Fine, S., Klein, E., Ambrus, J., Cohen, E., Derr, V. and Ambrus, C. "Interaction of Relatively Coherent Laser Radiation and Biological Systems," Federation Proceedings, 23, 1964.

15. Fine, S., Klein, E., Nowak, W.B., Hansen, W.P., Hergenrother, K., Scott, R.E. and Donoghue, J. "Measurements and Hazards on Interaction of Laser Radiation and Biological Systems," NEREM Record, 1958, 1964.

16. Nowak, W.B., Fine, S., Klein, E., Hergenrother, D., Hansen, W.P. "On the Use of Thermocouples for Temperature Measurement During Laser Irradiation," Life Sciences, 3:1495-1581, 1964.

17. Derr, V., Klein, E. and Fine, S. "Free Radical Occurrence in Some Laser Irradiated Biological Materials," Federation Proceedings, 24 (1), Supple. 14, Part III, January-February, 1965.

18. Fine, S., Klein, E., Nowak, W.B., Scott, R.E., Simpson, L., Crissey, J. and Donoghue, J. "Interaction of Laser Radiation with Biological Systems. I. Studies on Interaction with Tissues," Federation Proceedings, 24 (1), Suppl. 14, Part III, January-February, 1965.

19. Klein, E., Fine, S., Laor, Y., Simpson, L., Ambrus, J., Richter, W., Smith, G.K. and Aaronson, C. "Interaction of Laser Radiation with Biological Systems. II. Experimental Tumors," Federation Proceedings, 24 (1), Suppl. 14, Part III, January-February, 1965.

20. Klein, E., Fine, S., Ambrus, J., Cohen, E., Ambrus, C., Neter, I., Bardos, T. and Lyman, R. "Interaction of Laser Radiation with Biological Systems. III. Studies on *In Vitro* Preparations," Federation Proceedings, 24 (1), Suppl. 14, Part III, January-February, 1965.

21. Edlow, J., Fine, S. and Vawter, G.F. Federation Proceedings, 24:556, April, 1965.

22. Litwin, M., Fine, S., McCombs, H.L. and Klein, E. "Effects of Laser Radiation on the Surgically Exposed Canine Liver," Federation Proceedings, 24 (1), Suppl. 14, Part III, 566, March-April, 1965.

23. Fine, S., Klein, E., Fine, B.S., Litwin, M., Nowak, W.B., Hansen, W.P., Caron, J. and Forman, J. "Mechanisms and Control of Laser Hazards and Management of Accidents," Laser Technology Conference, April, 1965.

24. Laor, Y., Simpson, L.C., Klein, E. and Fine, S. "The Pathology of Laser Irradiation on the Skin and Body Wall of the Mouse," The American Journal of Pathology, Vol. 47, No. 4, October, 1965, pp. 643-63.

25. Hansen, W.P., Fine, S., Peacock, G.R. and Klein, E. "Focusing of Laser Light by Target Surfaces and Effects on Initial Temperature Conditions," NEREM Record, Vol. 7, 156-157, 1965.

26. Stratton, K., Pathak, M.A. and Fine, S. "ESR Studies of Melanin Containing Tissues After Laser Irradiation," NEREM Record, p. 150, 1965.

27. Klein, E., Laor, Y., Fine, S., Simpson, L.C., Edlow, J., Litwin, M. "Threshold Studies and Reversible Depigmentation in Rodent Skin," NEREM Record, Vol. 7, pp. 108-109, 1965.

28. Edlow, J., Fine, S., Vawter, G.F., Jockin, H. and Klein, E. "Laser Irradiation Effect on Rat Embryo and Fetus in Utero," Life Sciences, 4:615-23, 1965.

29. Klein, E. and Fine, S. "Biological Aspects of Laser Radiation." Abstract presented at the 149th National Meeting of the American Chemical Society, Detroit, Michigan, 1965.

30. Fine, S. and Klein, E. "Biological Effects of Laser Irradiation," in Advances in Biological and Medical Physics, Academic Press, 10:149-225, 1965.

31. Fine, S., Klein, E., Litwin, M., Peacock, G., Hamar, M. and Hansen, W.P. "Biological Effects of High Power Continuous N_2-CO_2 Laser Radiation at 10.6 Microns," Federation Proceedings, Vol. 25, No. 2, Part I, March-April, 1966.

32. Laor, Y., Hust, F., Fine, S. and Klein, E. "Studies on Biological Effects of Laser Radiation," Symposium on Biomedical Engineering, Marquette University, Milwaukee, Wisconsin, Vol. 1, pp. 316-318, June, 1966.

33. Lobene, R. and Fine, S. "Interaction of Laser Radiation with Oral Hard Tissues," Journal of Prosthetic Dentistry, 16:3, 589-597, May-June, 1966.

34. Fine, S., Klein, E., Litwin, M. "Laser Radiation and Therapy of Malignant Melanomas," New Views of Skin Diseases, Boston: Little, Brown & Co., 1966.

35. Fine, S., Klein, E., Fine, B.S., Hansen, W.P., Peacock, G.R. and Litwin, M. "Implementation of Procedures and Techniques for Safe Operation of Lasers," Proceedings of the First Conference on Laser Safety, November, 1966.

36. Klein, E., Fine, S., Laor, Y., Hust, F. and MacKeen, D. "Injurious Effects of Laser Radiation on Mammals," published in Proceedings of the First Conference on Laser Safety, November, 1966.

37. Fine, S. and Klein, E. "Ultraviolet Lasers," presented at the First Conference of the Biologic Effects of Ultraviolet Radiation, published in The Biologic Effects of Ultraviolet Radiation," F. Urbach, editor, Pergamon Press, 1969.

38. Fine, S., Hansen, W.P., Peacock, G.R., Klein, E., Hust, F. and Laor, Y. "Bio-physical Studies with the CO_2 Laser," NEREM Record, 8:166-167, November, 1966.

39. Fine, B.S., Fine, S. and Zimmerman, L.E. "CO_2 Lasers Irradiation on the Rabbit Eye, Clinical and Histopathologic Observations," NEREM Record, 8:160-161, November 1966.

40. Fine, S., Klein, E. "Biological Effects of Laser Radiation," in McGraw-Hill Yearbook of Science and Technology, 1966.

41. Klein, E., Fine, S., Laor, Y. and Hust, F. "Biological Effects of Laser Radiation," Proceedings American Society for Cancer Research, 1966.

42. Fine, S., Klein, E., Hansen, W.P. and Litwin, M. "Biological Effects of Laser Radiation," Digest of Technical Papers, International Quantum Electronics Conference, 1966.

43. Fine, S., Klein, E., Haynie, W.H., Litwin, M., Laor, Y. and Hust, F.S. "Biological Effects and Hazards of Laser Radiation," presented at American College of Physicians Conference, 1966.

44. Fine, S., Klein, E., et al. "Management Study on Biological Effects of Laser Radiation Program" for U.S. Air Force, Parts I, II, and III, 1000 pages, 1966.

45. Klein, E., Fine, S., Laor, Y., Hust, F., Litwin, M. and Knubbe, K. "Interaction of Laser Radiation with Experimental Melanoma," Proc. Am. Assoc. Cancer Res., 7:36, 1966.

46. Litwin, M.S., Fine, S., Klein, E., Fine, B.S. and Raemer, H. "Hazards of Laser Radiation Mechanisms, Control and Management," American Industrial Hygiene Assoc. Journal, 28:68-75, January-February, 1967.

47. Fine, S., Klein, E., Parr, W.H., Fine, B., Fisher, R.S., Peacock, G.R., MacKeen, D., Hansen, W.P. and Feigen, L. "Hazard Studies with Laser Radiation," Conference on Laser Technology, April, 1967.

48. Hardy, L.B., Hardy, F.S., Fine, S. and Sokal, J. "Effect of Ruby Laser on Mouse Fibroblast Culture," Federation Proceedings, 26:688, April, 1967.

49. Fine, B.S., Fine, S., Peacock, G.R., Geeraets, W. and Klein, E. "Preliminary Observations on Ocular Effects of High-Power, Continuous CO_2 Laser Irradiation," American Journal of Ophthalmology, Vol. 64, No. 2, pp. 209-222, August, 1967.

50. Peacock, G.R., Hansen, W.P. and Fine, S. "Increasing the Power Output from Inexpensive CO_2 Lasers," American Journal of Physics, Vol. 35, No. 8, 776-777, August, 1967.

51. Fine, S., Feigen, L., MacKeen, D. and Klein, E. "Hazards and Protective Devices Associated with 10.6 u Radiation," presented at Conference on Engineering in Medicine and Biology, 1967. Published in Proceedings, November, 1967.

52. Hansen, W.P., Feigen, L. and Fine, S. "A 'Worst Case' Analysis of Continuous Wave He-Ne Laser Hazards to the Eye," Applied Optics, Vol. 6, No. 11, pp. 1973-1975, November, 1967.

53. Laor, Y., Simpson, L., Klein, E., Fine, S. and Hust, F. "Effects of Laser Radiation on the Skin and Underlying Tissue of Mice during Controlled Hair Growth Cycle," Journal of Invest. Derm., 48:297-298, 1967.

54. Fine, S., Edlow, J., MacKeen, D., Feigen, L., Ostrea, E. and Klein, E. "Focal Hepatic Injury and Repair Produced by Laser Radiation: Pathologic and Biophysical Studies," American Journal of Pathology, Vol. 52, No. 1, pp. 155-176, January, 1968.

55. Hansen, W.P. and Fine, S. "Melanin Granule Models for Laser Induced Retinal Injury," Applied Optics, Vol. 7, No. 1, pp. 155-159, January, 1968.

56. Aaron, A., Fine, S. and Schetzen, M. "Safety Improvement for Unattended Lasers," Laser Focus, February, 1968.

57. Lobene, R.R., Raj Bhussry, B. and Fine, S. "The Interaction of Carbon Dioxide Laser Radiation with Enamel and Dentin," Journal of Dental Research, Vol. 47, No. 2, pp. 311-317, March-April, 1968.

58. Cohen, E. and Fine, S. "In Vitro Effects of Laser Irradiation on Human Gamma Globulin," Federation Proceedings, 27:1, 473, March-April, 1968.

59. Parker, G.S., Bavley, H.A. and Fine, S. "Report on Massachusetts Laser Survey," Laser Focus, 11:30-32, May, 1968.

60. Parker, G.S., Bavley, H., Fine, S., Powell, C. and Keene, B. "Laser Survey in Massachusetts," Health Physics Society Annual Meeting, Denver, Colorado, June 16-20, 1968. (Abstract.)

61. Fine, B.S., Fine, S., Feigen, L. and MacKeen, D. "Corneal Injury Threshold to Carbon Dioxide Laser Irradiation," Vol. 66, No. 1, pp. 1-15, July, 1968, American Journal of Ophthalmology.

62. Cohen, E., Klein, E. and Fine, S. "Effects of Laser Irradiation on Some Serologic Properties of Human Gamma Globulin," accepted for publication.

63. MacKeen, D., Fine, S. and Klein, E. "Toxic and Explosive Hazards Associated with Lasers," Laser Focus, pp. 47-49, October, 1968.

64. Fine, B.S., Berkow, J.W., Fine, S. "Corneal Calcification," Science, Vol. 162, pp. 129-130, October 4, 1968.

65. Hansen, W.P. and Fine, S. "Application of Thermal Models to Retinal Threshold Injury," presented at Laser Industry Association meeting, October 24-26, 1968, published in Proceedings of the Laser Industry Association Convention, 1968.

66. Bock, F., Laor, Y., Fine, S. and Klein, E. "Exploration of Potential Carcinogenic Effects of Pulsed Laser Radiation," presented at Laser Industry Association meeting, October 24-26, 1968, published in Proceedings of the Laser Industry Association Convention, 1968.

67. Fine, S., MacKeen, D., Feigen, L. and Fine, B. "Anterior Chamber Measurements on CO_2 Laser Corneal Irradiation," Proceedings of the Annual Conference on Engineering in Medicine and Biology, Vol. 10, p. 6, November 4, 1968.

68. Feigen, L., MacKeen, D. and Fine, S. "A Method for Detecting and Measuring Frequency of Surface Vibrations Using a Helium-Neon Laser," Review of Scientific Instruments, Vol. 40, pp. 381-382, February 2, 1969.

69. Geeraets, W., Fine, B.S. and Fine, S. "Ophthalmic Studies on CO_2 Laser Irradiation," Acta Ophthalmologica, (Koberhavn), Vol. 47, pp. 80-92, 1969.

70. Laor, Y., Simpson, C.L. Klein, E. and Fine, S. "Pathology of Internal Viscera Following Laser Radiation," American Journal of Medical Sciences, Vol. 257, pp. 242-252, April, 1969.

71. Litwin, M.S., Fine, S., Klein, E. and Fine, B.S. "Burn Injury After Carbon Dioxide Laser Irradiation," Arch. Surg., Vol. 98, pp. 219-222, February, 1969.

72. Fine, S. and Klein, E. "Lasers in Biology and Medicine," Laser Focus, pp. 28-36, July, 1969.

73. Fine, S., Bushor, W. and Cos, M. editors. Proceedings of the Laser Industry Association Meeting, October 24-26, 1968.

74. Fine, S. and Klein, E. "Lasers in Biology and Medicine" published in Development in Laser Technology, Society of Photo-Optical Instrumentation Engineers.

75. MacKeen, D., Fine, S., Feigen, L. and Fine, B.S. "Anterior Chamber Measurements on CO_2 Laser Radiation," Investigative Ophthalmology, Vol. 9, No. 5, pp. 366-371, May, 1970.

76. MacKeen, D., Edlow, J., Fine, S., Kopito, L. and Klein, E. "Calcium and Magnesium in Focal Hepatic Lesions," Federation Proceedings, Vol. 29, No. 2, March-April, 1970.

77. Klein, E., Laor, Y. and Fine, S. "Interaction of Laser Radiation with the Skin," Abstract-Laser Journal, Vol. 2, No. 1, January-February, 1970.

78. Fine, S., MacKeen, D., Berkow, J. and Fine, B.S. "Biological Studies with Laser Protective Materials," American Journal of Ophthalmology, Vol. 71, No. 4, April, 1971.

79. Campbell, J. and Fine, S. "Heat Sensation Thresholds for CO_2 Laser Radiation," Radiation Research, 43 (1), 1970.

80. MacKeen, D., Fine, S., Aaron, A. and Fine, B.S. "Cataract Production in Rabbits with an Ultraviolet Laser," Laser Focus, April, 1971.

81. Fine, S. and Hansen, W.P. "Optical Second Harmonic Generation in Biological Systems," Applied Optics, October, 1971.

82. MacKeen, D.L., and Fine, S. "Effect of Suprathreshold CO_2 Laser Irradiation of the Weanling Rabbit Eye on Lenticular Ascorbic Acid and Reduced Glutathione," Federation Proceedings, Vol. 32, No. 3, Pt. 1, 1973.

83. MacKeen, D., Fine, S. and Fine, B.S. "Production of Cataracts in Rabbits with an Ultraviolet Laser" - Ophthalmic Research, 5:317-324, 1973.

84. MacKeen, D., Cohen, J., and Fine, S. "Simultaneous Corneal Surface and Anterior Chamber Temperature Measurements on CO_2 Laser Irradiation," Federation Proceedings, Vol. 33, No. 3, Pt. 1, March, 1974. (Abstract.)

85. MacKeen, D.L., Szabo, G., and Fine, S. "The Effects of UV Laser Radiation at 325 nm on the Skin," The Yale Journal of Medicine, 1973 (Abstract).

B. Non-Laser Related

In addition, credit was given to non-laser related studies, in which the principal investigator and his associate were involved. A number of these were listed in the annual progress reports; several are listed below.

86. Fine, S., Klein, E., R.E., Hainish, H. and Aaronson, C. "Bio-Engineering in the Biological Sciences," IEEE Student Journal, January, 1964, 1:33-39.

87. Litwin, S.B., Cohen, J., Fine, S. and Aaron, A. "Rupture and Tensile Strength Measurements of Fresh and Teated Canine Aortic Tissue," Proceedings of the Annual Conference on Engineering in Medicine and Biology, Vol. 10, p. 44, November 4, 1968.

88. Aaron, A., Litwin, S.B., Fine, S. and Sillin, L. "Pressure and Flow Relations in Canine Aortic-Pulmonary Shunts," presented at Second International Conference on Medical Physics, Boston, Massachusetts, August, 1969, published in Abstracts of Conference.

89. Aaron, A., Litwin, S.B., Fine, S. and Rosenthal, A. "Determination of Cardiac Output by Dye Dilution," in Proceedings of the 23rd Annual Conference on Engineering in Medicine and Biology, Vol. 12, 1970.

90. Cohen, J., Litwin, S.B., Aaron, A. and Fine, S. "The Rupture Force and Tensile Strength of Canine Aortic Tissue," J. Surg. Research, December, 1972.

91. Litwin, S.B., Cohen, J. and Fine, S. "Effects of Sterilization and Preservation on the Rupture Force and Tensile Strength of Canine Aortic Tissue," J. Surg. Research, 1973.

C. Laser-Related Presentations as Invited Lecturer, Pertinent to Contract to Which Credit Was Given

1. Conference on Biological Effects of Laser Radiation, Washington, D.C. - Sponsored by U.S. Army Medical Research and Development Command, 1964

2. Conference on Lasers, New York Academy of Sciences, 1964

3. Gordon Research Conference on Biological Effects of Laser Radiation, 1965

4. Conference on the Biological Effects of Lasers, National Institutes of Health, Bethesda, Maryland, October 4-5, 1965

5. Gordon Research Conference on Biological Effects of Laser Radiation, 1966

6. Bell Telephone Laboratories - invited lecturer, 1966

7. The Martin Company - symposium on Biological Effects of Laser Radiation, 1966

8. Boston Medical Physics Society - Lecturer on Biophysical Studies with Laser Radiation, 1966

9. Seminar on Biological Effects of Laser Radiation, University of Texas, Austin, 1966

10. Conference on Development of Lasers in the Biological Sciences, Veterans Administration, Department of Medicine and Surgery, Washington, D.C., August 5, 1966

11. Presentation before the Physicians of the Association of American Railroads, Montreal, June 4, 1967

12. Gordon Research Conference on Biological Effects of Laser Radiation, 1967 (Session Chairman)

13. American College of Obstetrics - District I Meeting - Invited Participant - Biological Studies on Laser Radiation, October 1967

14. Invited Lecturer on Lasers - P.R. Mallory & Co. - Laboratory for Physical Sciences - Biological Effects of Laser Radiation, February, 1968

15. Brookhaven National Laboratory, Upton, New York "Biophysical Effects of Laser Radiation", May, 1968

16. New England Chapter Health Physics Society, "Biological Effects and Hazards of Laser Radiation", May, 1968

17. Case Western Reserve University, Cleveland, Ohio, Summer course on Laser Technology and Applications, presented lecture "Lasers in Biology and Medicine," July, 1968

18. G-APURSI Symposium (International Antenna and Propagation Symposium) "Electromagnetic Waves (Lasers) for Biological and Medical Applications", September, 1968

19. Rutgers University, New Brunswick, New Jersey, Participant in "Evaluation of Laser Hazards Course", October, 1968

20. S. Fine - "Biological Studies Relating to Laser Irradiation, Particulary with Respect to the Eye", Howe Laboratories, Massachusetts Eye and Ear Infirmary, Harvard Medical School, December, 1968

21. S. Fine - "Control of Laser Hazards and Management of Accidents", National Center for Radiological Health, U.S. Department of Health, Education and Welfare, Rockville, Maryland, February, 1969

22. S. Fine - "The Application of Lasers to Biology and Medicine," Conference on Trends and Directions in Biological Sciences of the Thirteen Colleges Curriculum Program Biology Teachers, Clark College, Atlanta, Georgia, March, 1969

23. S. Fine, Participation in Skin Laser Workshop, Second International Laser Safety Conference, Cincinnati, Ohio, March, 1969

24. S. Fine, Lasers--Characteristics, Use, Hazards and Biological Effects, Seminar Series, Environmental Health Engineering and Science, Graduate School of Engineering, Northeastern University, March, 1969

25. S. Fine, Lasers--Characteristics and Uses in Biology and Medicine, Surgical Seminar Series, Boston University School of Medicine, March, 1969

26. S. Fine, Use of Lasers in Biology and Medicine, Laser Applications Course, Washington University, St. Louis, Missouri, May, 1969

27. E. Klein, and S. Fine, Tissue and Cell Effects of Laser Radiation--Gordon Research Conference on Lasers in Medicine and Biology, June, 1969

28. S. Fine, "Lasers in Biomedicine," I.E.E.E. Student Branch, Northeastern University, July, 1969

29. S. Fine, "Biological Hazards and Effects of Laser Radiation," in course on Fundamentals of Non-Ionizing Radiation Protection, Northeastern Radiological Health Laboratory, August, 1969

30. S. Fine, Lasers in Industry, Associated Hazards and Protection, National Safety Congress, Chicago, Illinois, October 28, 1970

31. S. Fine, Non-invasive Testing in Medicine, I.E.E.E. group on Engineering in Biology and Medicine, Boston, Massachusetts, November, 1970

32. S. Fine, Uses and Hazards of Laser Radiation in Industry and in Atmospheric Pollution Studies, 24th AMA Clinical Convention, Boston, Massachusetts, November 30, 1970

33. S. Fine, "Bioengineering", Massachusetts Epsilon Chapter, Tau Beta Pi (Northeastern University), September, 1969

34. S. Fine, "Laser Biology", in Laser Fundamentals and Applications course, Polytecnnic Institute of Brooklyn Graduate Center, September, 1969

35. S. Fine and E. Klein, "Biological Effects of Laser Radiation," the Theobald Smith Society, New Jersey, October, 1969

36. S. Fine, "Lasers--Biological Effects and Medical Applications," Society of Photo-optical Instrumentation Engineers Meeting, co-sponsored by the University of Rochester Institute of Optics, Rochester, New York, November, 1969

37. S. Fine, Session Chairman, Laser and Ultraviolet Contributed Papers, Fourth Annual Midyear Topical Symposium, Health Physics Society Meeting, Louisville, Kentucky, January 28-30, 1970

38. S. Fine, Lasers in Industry, Associated Hazards and Protection, National Safety Congress, Chicago, Illinois, October 28, 1970

39. S. Fine, Non-invasive Testing in Medicine, I.E.E.E. group on Engineering in Biology and Medicine, Boston, Massachusetts, November, 1970

40. S. Fine, Uses and Hazards of Laser Radiation in Industry and in Atmospheric Pollution Studies, 24th AMA Clinical Convention, Boston, Massachusetts, November 30, 1970

41. S. Fine, "Lasers in Biology and Medicine," in course on Lasers and Optics for Applications, Massachusetts Institute of Technology, Cambridge, Massachusetts, July 30, 1971

42. S. Fine, Guest Lecturer in Graduate Course 2.77, "Biological Effects and Medical Applications on Non-Ionizing Radiation," Fall, 1971, Massachusetts Institute of Technology

43. S. Fine, "Medical Applications, Research and Safety," Boston Section I.E.E.E. 1972 Lecture Series, February, 1972

44. S. Fine, "Lasers in Biology and Medicine," a course on lasers and optics for application, M.I.T., July, 1972

45. S. Fine, "Biological Effects and Medical Applications on Non-Ionizing Radiation," guest lecturer for several sessions in graduate course 2.77, M.I.T., 1972-1973

46. S. Fine, "Biological Effects and Medical Applications of Non-Ionizing Radiation," guest lecturer in graduate course at M.I.T., 1973-9974

47. S. Fine, lectured at Raytheon Research Laboratory on Electrical Hazards and Emergency Management of Accidents, 1974

48. S. Fine, "Biological Effects and Medical Applications of Non-Ionizing Radiation", guest lecturer in summer session course, M.I.T., July, 1975

Most other conferences in which abstracts or papers were published are included in the preceding bibliography.

D. Laser-Related Conference Organization and Planning; Pertinent to Contract

1. Boston Laser Conference, 1963

2. Boston Laser Conference, 1964

3. Institute of Electrical and Electronics Engineers - Member, NEREM Program Committee, 1965

4. Institute of Electrical and Electronics Engineers - Member, NEREM Program Committee, 1966

5. American Association for the Advancement of Science - Session Organizer and Chairman of Session on Biological Effects of Laser Radiation, 1966

6. Laser Industry Association Convention, October 24-26, 1968

7. Course on "Fundamentals of Laser Radiation Protection" given to personnel of U.S. Department of Health, Education and Welfare, 1968

8. Member, program planning committee on seminar series in applications of physical chemical techniques in Biology and Medicine, EMB, IEEE, Boston Section, 1969

9. Laser Industry Association Meeting - Los Angeles, California, October 20-22, 1969

10. Electro-Optical System Design Conference, September 22-24, 1970, New York Coliseum. Planning of sessions, session organization and chairman.

11. Major participant in the organization, planning, and instruction of personnel, and field work related to the first major survey on lasers and laser devices in the United States which was carried out be the State of Massachusetts and Occupational Health and Radiological Health, H.E.W., 1968.

PERSONNEL RECEIVING CONTRACT SUPPORT AND GRADUATE DEGREES OBTAINED

Arnold Aaron	Ph.D. in Engineering
Charles Aaronson	M.S. in Engineering
John Campbell	M.A. in Psychology
John Caron	M.S. in Engineering
Joel Cohen	M.S. in Biology
John Donoghue	M.S. in Engineering
Larry Feigen	M.S. in Physics
James Forman	M.S. in Engineering
Peter Hansen	Ph.D. in Engineering
Karl Hergenrother	Ph.D. in Engineering
Donald MacKeen	Ph.D. in Biology

Note: The above individuals were supported in full or in part for contract related work while carrying out graduate work. In some cases, the support was minimal.

OTHER NON-GRADUATE DEGREE PERSONNEL RECEIVING SUPPORT INCLUDE:

James Blout
Francis Daniels
Evelina Gorman
Mary Laananen
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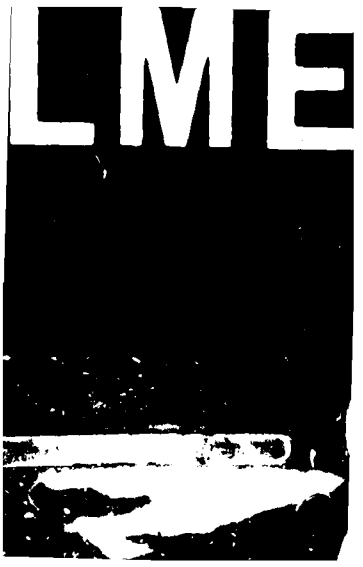
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